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FURTHER OBSERVATIONS ON THE FUNCTION OF THE GALL BLADDER

Experiments with Methylene Blue on the Dog[1]

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In a series of experiments on rabbits, Halpert and Hanke (1) have shown that following oral administration of methylene blue (in doses of 20 mgm. per kilogram of body weight) the dye ceased to be excreted by the liver at about the thirtieth hour, but remained in the gall bladder about forty-two hours longer. They concluded from their experiments that, in the rabbit, bile does not under ordinary conditions leave the gall bladder through the cystic duct. Their method offers possibilities of studying the mechanism of the biliary vesicle under the conditions of every day life. Since information of this kind is not available in the dog, it seemed desirable to gather data in this animal, which has so frequently been used for studies of the physiology of the biliary system.

Method. Four series of experiments were performed: a, acute experiments with injection; b, feeding experiments; c, injection experiments, and d, injection experiments with ligation of a hepatic duct. In all of the experiments the methylene blue content of the bile was determined quantitatively by the method devised by one of us (M. T. H.) (1).

a. Acute experiments with injection. The animal was anesthetized with ether, the abdomen was shaved, then opened, and a glass cannula tied into the ductus choledochus close to its termination in the duodenum. A short rubber tube connected this with a graduated pipette of 1 cc. capacity. After 1 cc. of bile had been collected, the amount of methylene blue calculated for the weight of the animal (20 mgm., i.e., 2 cc. of a 1 per cent solution of methylene blue per kilogram of body weight) was injected into the right saphenous vein. The time that elapsed between the injection and the

[1] This work has been conducted under the joint auspices of the Otho S. A. Sprague Memorial Institute and the Douglas Smith Foundation for Medical Research.

gross appearance of the dye in the pipette was noted, the bile was collected in 1 cc. portions, the rate of flow recorded and the methylene blue content determined quantitatively.

The results obtained are charted in table 1 and may be summarized as follows: 1. Injected intravenously, methylene blue appeared in the bile in from three to thirty-two minutes. 2. The highest concentration of the dye in the bile collected from the ductus choledochus was usually reached within one to two hours, and varied between 1:220 and 1:1,200. Values obtained about two and five hours following the injection were 1:240, 1:680, and 1:890, 1:1,700 respectively. 3. The bile removed from the gall bladder at the same time usually contained about one to two times as much methylene blue as that contained in the sample of bile obtained coincidently from the ductus choledochus.

TABLE 1

Summary of data on the excretion of methylene blue by the biliary system of the dog following intravenous administration of the dye in doses of 20 mgm. (i.e., 2 cc. of a 1 per cent solution) per kilogram of body weight

DOG	FIRST APPEARANCE OF DYE IN THE BILE	HIGHEST CONCENTRATION OF DYE IN THE BILE FROM DUCTUS CHOLEDOCHUS		DURATION OF EXPERIMENT		AVERAGE FLOW OF BILE PER HOUR	CONCENTRATION OF DYE IN BILE AT CLOSE OF EXPERIMENT		
		Reached within	Dilution				From D. choledochus	From gall bladder	Ratio of concentr. approx.
	minutes	*hours*		*hours*	*minutes*	*cc.*			
I-3	30	2	1:220	2	45	2.66	1:240	1:200	1:1
I-1	3	1	1:510	2	15	2.17	1:680	1:450	1:1.5
I-2	32	?	1:890	5	10	0.82	1:890	1:390	1:2.2
I-4	9	2	1:1,200	5		1.56	1:1,700	1:2,000	1:1

It is worthy of note that methylene blue colored the saliva about the time that it appeared in the bile and was invariably present in the gastric and duodenal contents at the close of the experiment, although bile did not pass into the duodenum through the common bile duct during the experiment.

b. Feeding experiments. In this series of experiments methylene blue was given by stomach tube. The amount administered was identical with that used in the experiments with injections (namely, 20 mgm. per kilogram of body weight); the operative procedure was also identical. After the administration of the dye the animals were returned to their cages and remained on their usual diet (consisting of chopped raw meat, milk, and bread given twice daily) until the time of operation. This was performed at stipulated intervals between the twenty-fourth and forty-eighth hour following the administration of the dye.

The results are charted in table 2 and may be summarized as follows: 1. In the twenty-four-hour group of six animals, methylene blue was always present in the bile removed from the gall bladder. The concentration of the dye varied from 1:760 to 1:14,200. 2. In the thirty-hour group of six animals methylene blue was present in the bile removed from the gall bladder in two, in concentrations of 1:2,900 and 1:7,000. Methylene blue could be determined in the bile collected from the ductus choledochus in only one of these animals. 3. In the forty-eight-hour group of six dogs, methylene blue was not present in the bile removed from the gall bladder.

The above data indicate that following oral administration of methylene blue, the dye disappears from the bile of the liver at about the thirtieth

TABLE 2

Concentration of methylene blue in the bile collected from the cannulated ductus choledochus and in the bile removed from the gall bladder of the dog, 24, 30, and 48 hours following oral administration of 20 mgm. (i.e., 2 cc. of a 1 per cent solution) of methylene blue per kilogram of body weight

DOG	D. CHOLEDOCHUS	GALL BLADDER	URINE
24-hour group			
F-5	—	1:760	—
F-2	—	1:820	—
F-3	—	1:1,240	—
F-4	—	1:1,250	—
F-6	—	1:1,370	—
F-1	—	1:14,200	—
30-hour group			
F-9	1:4,000	1:2,900	Trace
F-7	None	1:7,000	Trace
F-10	None	None	Trace
F-8	None	None	None
F-11	None	None	None
F-12	None	None	None
48-hour group			
F-13	None	None	None
F-14	—	None	None
F-15	—	None	None
F-16	—	None	None
F-17	—	None	None
F-18	—	None	None

hour, and from the gall bladder some time between the thirtieth and forty-eighth hour after ingestion. These facts suggested an inadequate resorption of methylene blue from the gastro-intestinal tract. In order to reduce this variable, a series of experiments was carried out with the intravenous method of administration.

c. Injection experiments. In this series of experiments methylene blue was given intravenously, usually through the right saphenous or femoral vein, in amounts identical with those used in the feeding experiments (namely, 20 mgm. per kilogram of body weight). After the injection of the dye the animals were returned to their cages and remained on their usual diet, as in the feeding experiments, until the time of operation. This was performed at stipulated intervals between the thirty-sixth and sixtieth hour following the injection of the dye.

The results are charted in table 3. From these we learn that in the thirty-six hour group of ten dogs the bile from the ductus choledochus contained methylene blue in six, viz., in concentrations of between 1:6,500 and 1:48,000 (in four) and traces (in two). The gall bladder on the other hand contained methylene blue in all but one of these animals, in concentrations varying from 1:4,000 to 1:38,600 (in seven) and traces (in two). In the forty-two hour group of six animals the bile from the ductus choledochus contained a trace of methylene blue in one of the animals and none in the others; the bile from the gall bladder contained the dye in two animals in concentrations of 1:2,400 and 1:5,500 respectively. In the forty-

TABLE 3

Concentration of methylene blue in the bile collected from the cannulated ductus choledochus and in the bile removed from the gall bladder of the dog, 36, 42, 48 and 60 hours following intravenous administration of 20 mgm. (i.e., 2 cc. of a 1 per cent solution) of methylene blue per kilogram of body weight

DOG	D. CHOLEDOCHUS	GALL BLADDER	URINE
36-hour group			
I-2	1:19,700	1:4,000	1:3,600
I-7	1:6,500	1:4,400	1:18,300
I-6	1:19,600	1:5,500	Trace
I-5	None	1:8,400	None
I-8	—	1:16,500	None
I-10	1:48,000	1:25,000	Trace
I-1	Trace	1:38,600	Trace
I-9	None	Trace	1:8,900
I-3	Trace	Trace	Trace
I-4	None	None	None
42-hour group			
I-15	None	1:2,400	1:15,500
I-11	Trace	1:5,500	1:9,900
I-13	None	None	None
I-14	None	None	Trace
I-12	None	None	None
I-16	None	None	None
48-hour group (a) Satisfactory feeding			
I-21	None	1:4,600	Trace
I-20	None	1:7,600	None
I-22	None	1:12,500	None
I-24	None	Trace	None
I-23	None	Trace	None
I-17	None	None	None
I-19	None	None	None
I-18	None	None	None
I-25	None	None	None
I-26	None	None	None
48-hour group (b) Unsatisfactory feeding			
I-27	1:1,820	1:1,040	None
I-28	None	1:3,900	Trace
I-29	Trace	1:7,500	Trace
I-31	None	1:7,800	Trace
I-30	None	1:8,000	None
I-32	1:13,000	1:11,000	None
60-hour group			
I-33	None	1:18,700	None
I-42	None	Trace	None
I-36	None	None	Trace
I-34	None	None	None
I-35	None	None	None
I-37	None	None	None
I-38	None	None	None
I-39	None	None	None
I-40	None	None	None
I-41	None	None	None

eight hour group of sixteen dogs ten were eating regularly while six had an unsatisfactory feeding record. The bile from the ductus choledochus of the first group contained no methylene blue, the bile from the gall bladder on the other hand contained the dye in five, in concentrations from 1:4,600 to 1:12,500 (in three) and traces (in two). The bile from the ductus choledochus of the second group (with the unsatisfactory feeding records) showed methylene blue in half of the animals (admixture from the gall bladder?), the bile in the gall bladder contained the dye in all of these animals in concentrations between 1:1,040 and 1:11,000. In the sixty-hour group of ten dogs the bile from the ductus choledochus did not

TABLE 4

Concentration of methylene blue in the bile removed from the gall bladder of the dog, 18 hours following intravenous administration of 20 mgm. (i.e., 2 cc. of a 1 per cent solution) of methylene blue per kilogram of body weight, and again 102 hours following ligation of a hepatic duct (incomplete obstruction), or the common bile duct (complete obstruction)

DOG	CONCENTRATION OF METHYLENE BLUE IN THE BILE FROM THE GALL BLADDER		OBSTRUCTION
	18 hours following intravenous administration	102 hours later, following ligation	
L-5	1:350	1:80,000	Incomplete
L-3	1:400	1:1,430	Incomplete
L-6	1:600	1:6,000	Incomplete
L-1	1:1,000	1:900	Incomplete
L-4	1:1,000	1:90,000	Incomplete
L-2	1:1,150	None	Incomplete
L-7	1:650	1:7,500	Complete
L-8	1:2,000	1:850	Complete
L-9	1:3,000	1:5,000	Complete

contain methylene blue, the bile from the gall-bladder contained the dye in a concentration of 1:18,700 in one animal and in traces in another. It is worthy of note that the dog in the sixty-hour group which showed a concentration of 1:18,700 of methylene blue in the bile from the gall bladder, had a perfect feeding record, i.e., it had eaten all six meals that were offered to him. The last two of these were given twelve and four hours before the operation. In other words, the dye had remained in the gall bladder of this dog, in spite of several feedings, at least eighteen hours after it had disappeared from the bile of the liver.

According to the above data methylene blue disappears from the bile of the liver some time about the thirty-sixth hour following its intravenous administration, and from the gall bladder between the thirty-sixth and sixtieth hour. In other words, a more or less complete exchange of the content of the gall bladder occurs within about twenty-four hours.

d. Injection experiments with ligation of the right hepatic duct. The purpose of the following experiments was to establish the route by which methylene blue was leaving the gall bladder. The usual amount of methylene blue (i.e., 20 mgm. per kilogram of body weight) was given intravenously to nine dogs. Eighteen hours later the hepatic duct, below the orifice of the cystic duct (in six dogs) or the common bile duct (in three dogs) was ligated, aseptically, and a sample of bile removed from the gall bladder. Five days after the injection of the dye, the animals were reopened, the condition of the biliary system noted, and bile aspirated from the gall bladder, and its methylene blue content determined.

The results (table 4) were about the same when some of the bile was still reaching the duodenum (ligation of the hepatic duct below the orifice of the cystic duct) or when no bile entered the duodenum (ligation of the common bile duct), and bring out the fact that methylene blue is resorbed from the dog's gall bladder rather slowly.

COMMENT. Reasons can easily be offered for explaining the divergence of views regarding the purpose and functions of the biliary vesicle (2). Perhaps the most obvious of these are that investigators have worked on different kinds of animals paying little or no attention to their feeding habits, the morphological differences of the biliary system and the possible individual variations; furthermore, maximal stimuli were usually employed under extraordinary conditions. Our aim was to gather information on the performances of the gall bladder under conditions of every day life. We believe that experiments with methylene blue furnish such data because the intravenous administration of this dye, in doses applied by us, is not followed by any constitutional symptoms and because our animals ate and lived the same way after as before the injection of the dye.

The acute experiments with injection informed us of the first appearance of the dye in the bile following its intravenous administration (3 to 32 minutes), and of the great individual variations of the highest concentration attained in the bile of the liver, 1:220 to 1:1,200. It is of particular interest that the dye colored the saliva and was invariably present at the close of the experiment (two to five hours after the injection) in the gastric and duodenal contents, although bile did not pass into the duodenum from the common bile duct at any time during the experiment. These facts should caution against hasty conclusions as to the site of origin of methylene blue when discovered in the duodenum a number of hours following intravenous administration of the dye (3).

The results of the feeding experiments show that the early disappearance of methylene blue from the bile, following its oral administration, is due to an inadequate resorption of the dye from the gastro-intestinal tract.

The results of those experiments in which after intravenous administration of the dye, the animals remained in their usual environment and diet

until the time of operation, are illuminating. They show that the liver ceased to produce bile containing methylene blue at about the thirty-sixth hour following its intravenous administration. The gall bladder at this time contained methylene blue in about 70 per cent of the dogs. Methylene blue usually vanished from the gall bladder from six to twenty-four hours after the liver ceased to produce bile containing the dye. This indicates that approximately twenty-four hours are necessary for a more or less complete exchange of the content of the gall bladder. The fact that the methylene blue content of the bile in the gall bladder of the dogs with unsatisfactory feeding records (48-hour group (b)), was invariably high, suggests that the exchange of the content of the gall bladder is hastened by the ingestion and digestion of food.

The injection experiments with ligation of the hepatic duct (distal to the orifice of the cystic duct) or the common bile duct, show that under such circumstances methylene blue is not resorbed from the gall bladder with sufficient rapidity to account for a more or less complete exchange of the content of the viscus within twenty-four hours. In other words, it is necessary to assume that a great deal of dye left the gall bladder by the way of the cystic duct. These injection experiments with ligation also show that the concentration of methylene blue in the gall bladder is about the same whether some of the bile is still reaching the duodenum (ligation of the hepatic duct below the orifice of the cystic duct), or when bile does not enter the duodenum (ligation of the common bile duct). They also further illustrate the great individual variations in the excretory functions of the liver and the resorptive performances of the gall bladder.

A study is under way attempting to correlate the variations in the performances of the biliary vesicle with individual differences in the morphology of the extra-hepatic biliary ducts and the gall bladder.

SUMMARY

1. Methylene blue given intravenously (in doses of 20 mgm., i.e., 2 cc. of a 1 per cent solution per kilogram of body weight) to dogs anesthetized with ether, appears in the bile within thirty minutes, and attains its highest concentration of 1:220 to 1:1,200 within one to two hours. The dye also appears in the saliva, and the gastric and duodenal secretions.

2. In intact dogs, methylene blue ceases to be present in the bile of the liver about the thirty-sixth hour following its intravenous administration; from the content of the gall bladder it usually vanishes from six to twenty-four hours after the liver ceased to produce bile containing the dye.

3. After oral administration, methylene blue disappears from the bile of the liver at about the thirtieth hour, and from the gall bladder some time between the thirtieth and forty-eighth hour after ingestion.

4. Exchange of the content of the gall bladder occurs within about twenty-four hours; this process is retarded when feeding is unsatisfactory.

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A COMPARISON OF THE EFFECTS OF LOW ALVEOLAR OXYGEN, OF SODIUM CYANIDE AND OF SODIUM SULFIDE UPON RESPIRATORY MOVEMENTS AND THE RESPONSE OF MUSCLE TO DIRECT, INDIRECT AND REFLEX STIMULATION[1]

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Dogs were used which weighed from ten to thirty kilos. They were anesthetized by injection of 60 mgm. of morphine sulfate and 0.8 gram of urethane per kilo body weight. The trachea was connected to a rebreathing tank of one hundred liters capacity from which the carbon dioxide was absorbed by soda-lime. Blood pressure was recorded from the common carotid artery and injections were made into the external jugular vein.

Double pneumothorax was produced by an incision between two ribs into the thoracic cavity and a steel tube one and one-quarter inch i.d. inserted. This was done to allow constant artificial ventilation. By withdrawing the air from the thoracic cavity and plugging the steel tube the animal could be allowed to breathe naturally again. During artificial ventilation respiratory movements were recorded by means of a lever attached to the abdominal muscles.

To record reflex contractions of the anterior tibialis muscle the tendon was isolated at its insertion, severed, freed from surrounding fascia for a distance of three to five centimeters and connected by a cord running across freely moving pulleys to a muscle lever. The sciatic nerve was then isolated, the sensory trunk severed and a shielded electrode applied central to the cut end. The motor nerve was isolated at the lateral side of the knee, severed and placed in a shielded electrode for indirect stimulation of the muscle. To stimulate the sartorius muscle directly an electrode consisting of a $\frac{1}{2}$ inch $\times$ 3 inch piece of thin bakelite on which two parallel platinum strips were fastened lengthwise was placed beneath the isolated, severed end of the muscle in such a manner that the muscle fibres stretched at right angles across the platinum strips. The femoral nerve was severed near the inguinal ligament to avoid any reflex stimu-

[1] Preliminary report appeared in the Proceedings of the Society for Experimental Biology and Medicine, 1929, xxvi.

lation of the muscle. Obviously but two muscles could be used simultaneously.

Muscular contractions were recorded by two muscle levers which measured eight and one-half inches from the fulcrum to the writing tip. During submaximal stimulation the cord was attached one inch from the fulcrum. During supermaximal stimulation, because of the greatly increased contractions the attachment was moved to a point four inches from the fulcrum, therefore comparisons of changes produce during submaximal or supermaximal stimulation must take into account the leverages employed.

Break shocks of an induction coil were delivered by a rotating stimulus selector. The stimulus required for each muscle was regulated by approximating or separating the respective secondary and primary coils. Both submaximal and supermaximal stimuli were used.

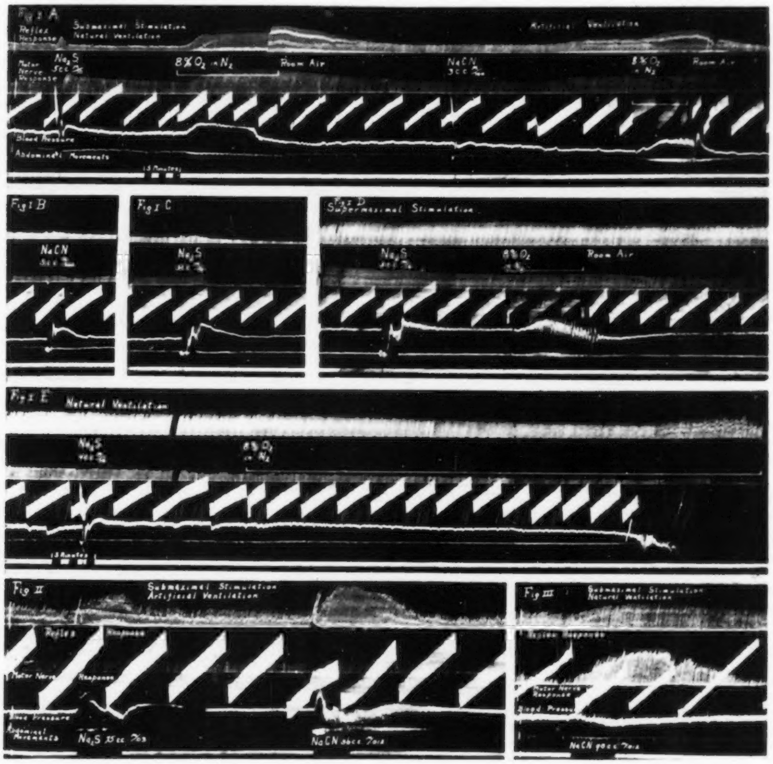
Results. Records published under the same figure numbers (e.g., figs. I A, B, C, D, E) are from the same experiment. Long normal periods have been eliminated in such records to conserve space.

I. *Submaximal stimulation during normal variable ventilation.* The response of the muscle reflexly stimulated was usually increased during administration of mixtures low in oxygen content (fig. I A). On one occasion there was no increase but rather a decreased response from the beginning of the administration to the end and an increase to normal on readministration of room air. In a few instances there was no change in the response during administration of low percentages of oxygen. In some cases the only change was a late decrease in response. Occasionally there was an initial increase with administration of mixtures low in oxygen which declined progressively to the initial level and even below accompanied by failing respiration. This response returned momentarily to a much higher level than normal with readministration of room air (fig. VIII). The muscle stimulated through its motor nerve, commonly showed an increase in response during administration of mixtures low in oxygen (fig. I A, VI A, VII, VIII). On one occasion there was a decrease in response and in a few instances no change occurred.

When the muscle was directly stimulated there was also a common increase in response during administration of gaseous mixtures low in oxygen content. In one instance this increased response was comparable to the simultaneous increase in response of the muscle stimulated through its motor nerve (fig. VI A). Such large changes, however, were the exception. Figure IV A illustrates a more usual response—a gradual increase until the end of the administration.

If injected rapidly sodium cyanide caused an hyperpnea followed by apnea. If more slowly injected, respiration was stimulated over a long period without being paralyzed. Sodium cyanide rapidly injected was

almost invariably accompanied by an increased reflex response. Figure I A is an exception. The muscle stimulated through its motor nerve also commonly showed increased response during injection. The muscle directly stimulated sometimes showed increased response during injection but more frequently showed no change in response. When injected slowly



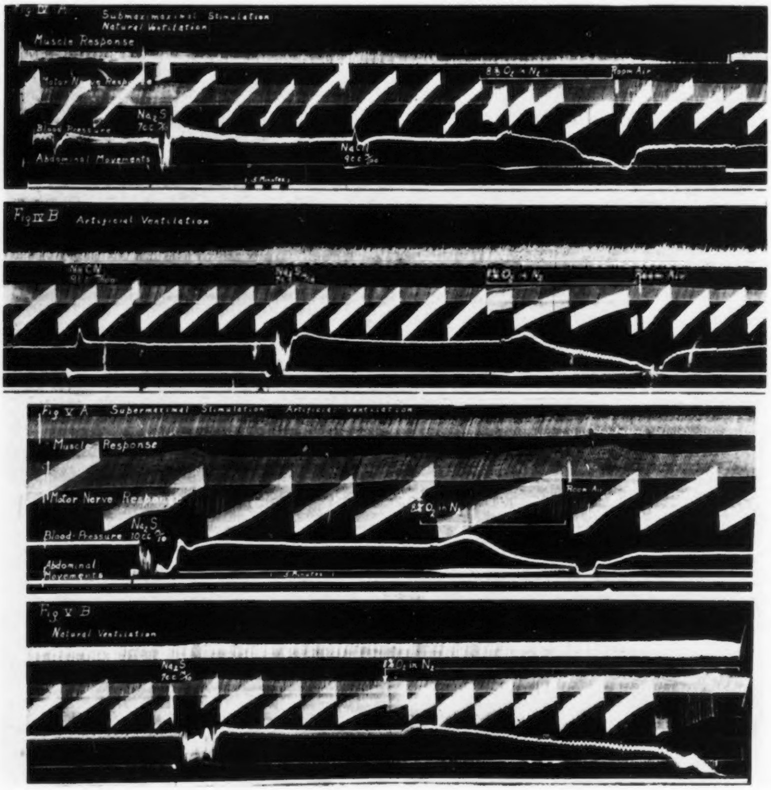
Figs. I-III

the response was usually less pronounced but always in the same direction and continued as long as the injection (fig. III).

Rapid injection of sodium sulfide was accompanied by greatly augmented respiration followed by an apnea with a later return to normal ventilation. Rapid injections were almost always accompanied by increased response of the muscle reflexly stimulated and of the muscle stimulated through its motor nerve (fig. I A). The muscle stimulated

directly usually showed no change. When a changed response did occur it was an increase. With longer and slower injections there was a period of augmented respiration but no apneic period at the end of the injection. The effects in general were similar but of greater magnitude.

II. *Submaximal stimulation during artificial constant ventilation.* Administration of low oxygen mixtures, sodium cyanide and sodium sulfide

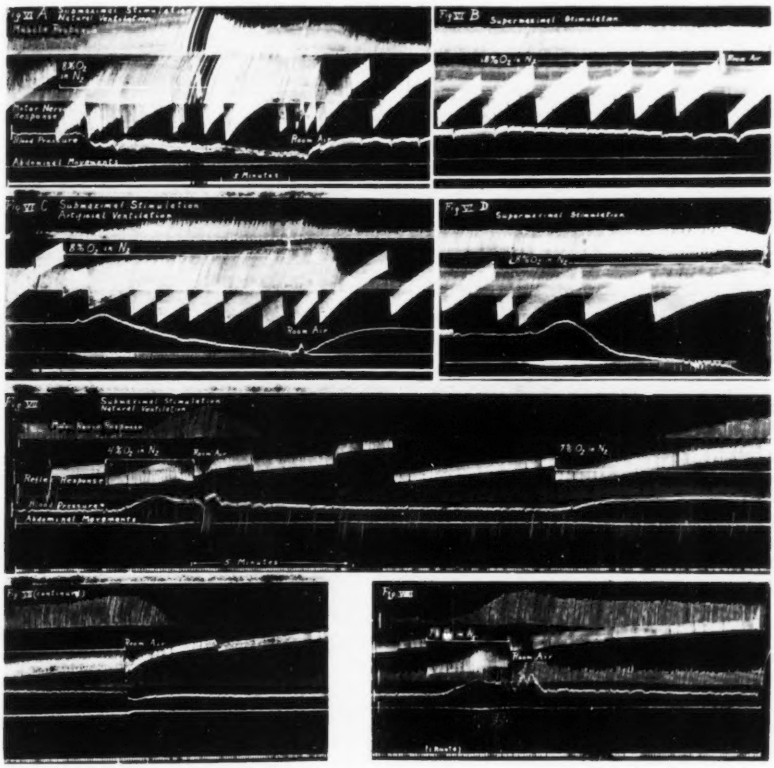


Figs. IV–V

during constant pulmonary ventilation gave the same general results as those during natural variable ventilation. (See figs. I A, I B, I C, II, IV B, VI C.)

Supermaximal stimulation. Whether ventilation was natural or artificial the administration of oxygen poor mixtures during supermaximal stimulation resulted usually in no change in response to reflex stimulation

or stimulation through the motor nerve or stimulation directly. Sometimes there was a small increase in response with each form of stimulation (figs. I D, V A, V B, VI B, VI D) and in a very few instances there was a decrease in response with each form of stimulation. The persistence of the same height of contraction throughout oxygen low mixtures was, however, the usual result.



Figs. VI–VIII

On the whole the results were the same for sodium cyanide and sodium sulfide. In a few instances there was an increase in response with all forms of stimulation during either rapid or slow injection and a few times there was a decrease in response with all forms of stimulation during either type of injection.

When administrations of oxygen poor mixtures, sodium cyanide or sodium sulfide were continued until death there was a much greater tend-

ency to increased response to all three types of stimulation whether the stimulus was submaximal or supermaximal (figs. I E, V B, VI D).

Discussion. The results of these experiments clearly indicate a rough parallelism between the response of the respiratory movements and simple muscular contractions elicited by submaximal stimulation (direct, indirect and reflex) on the administration of gaseous mixtures low in oxygen and on the intravenous injection of sodium cyanide and sodium sulfide. The parallelism points to an accessory augmenting chemical control of pulmonary ventilation operating through cells other than those in the respiratory center proper. Whether this chemical augmentation exerted through outlying cells is similar in nature requires further study.

The possibility of impairment of oxidation in the respiratory center being associated with increasing acidity introduces the difficulty of clearly separating the effects of acidity and of impairment of oxidations independent of pH changes on pulmonary ventilation. The separation of these effects, however, seems more evident with other physiological functions. The secretion of cerebrospinal fluid (Nicholson, 1929) and of saliva (Eddy, 1929) is augmented by carbon dioxide administration and slowed by administration of gaseous mixtures low in oxygen. Muscle response to the three types of stimulation here employed, on the other hand, is augmented by administration of gaseous mixtures low in oxygen and definitely inhibited by the administration of carbon dioxide.

The results of the present experiments on muscle response might be interpreted in two ways. Impaired oxidations might exert an augmenting action independent of hydrogen ion concentration or the excessive ventilation accompanying hyperpnea might overcompensate the acid effect of anaerobic metabolism and augment the muscular response by turning the tissues alkaline. The first interpretation is in line with the view that impaired oxidations independent of acid change may be an important stimulating factor in the control of ventilation. For that reason the effects of impaired oxidation on muscle in the absence of hyperventilation were studied by administration of constant artificial ventilation. It was believed that under such condition the tissues would unquestionably turn acid. The experiments of Gesell, Krueger, Gorham and Bernthal (1930) done under these conditions indicate a general increase in tissue acidity. The augmentation of muscle response during impaired oxidations with constant pulmonary ventilation, therefore, indicates an excitatory effect of impaired oxidations which more than compensates the inhibitory effect of increased acidity.

Recent quantitative experiments (Gesell, Brassfield, Krueger, Nicholson, 1930) done on the intact animal in which ventilation is under normal control indicate a decrease in hydrogen ion concentration of resting muscle and of the testicle on the administration of gaseous mixtures low in oxygen.

Assuming similar changes in the spinal cord, nerves and muscles of the present experiments during hyperpnea of lowered alveolar oxygen it is then conceivable that the augmented muscle response under these conditions is a resultant of two effects—decreased hydrogen ion concentration and impaired oxidations working in the same direction. There is some evidence from the records of these experiments, to indicate that such is actually the case. Thus in figure I A during normal variable ventilation when the administration of low alveolar oxygen was accompanied by a marked hyperpnea the response of the muscle reflexly stimulated was noticeably greater than it was later in the experiment when the same oxygen mixture was administered artificially. In the latter instance very probably increasing acidity counteracted the stimulating effect of lowered oxidations.

These experiments thus suggest the existence of a peripheral chemical control of respiration inherent in at least four distinct structures outside of the respiratory center; muscle, motor and sensory nerve and reflex center. The parallelism between changes in respiratory movements and changes in reflex response is marked as is seen in figures VII and VIII. The response of the muscle indirectly stimulated, however, is very different. The failing of response is missing. Since the muscle directly stimulated also showed changes in response during lowered oxidations it may be supposed that muscle tissue may itself modify the impulses arriving at it. During supermaximal stimulation as has been shown there was frequently a small increase in response to all three types of stimulation when oxidations were lowered. From the nature of the stimulus it is obvious that these changes were probably due to changes in contractility of the muscle fibers. But since the changes in response of the muscle directly stimulated with supermaximal stimuli were always definitely less than when a submaximal stimulus was applied it follows that some of the changes observed during submaximal stimulation were due to changes in irritability.

SUMMARY

The contractions of the anterior tibialis muscle when stimulated reflexly, indirectly through its motor nerve, and directly was recorded during the administration of sodium cyanide, sodium sulfide and gaseous mixtures low in oxygen.

When submaximal induction shocks were used there was a definite increase in contraction to each type of stimulation. When supermaximal stimuli were employed the effects were missing or small.

Since these results are obtained during constant ventilation when the tissues are assumed to turn acid it is concluded that the general increase

in excitability of the muscle, nerve, and cord occurs independently of acid changes for carbon dioxide produces a general decrease in excitability.

It is concluded that impairment of oxidations may result in a chemical control of ventilation peripheral to the respiratory center proper by action on sensory nerve fibers, by central action on the cord, by action on sensory nerve fibres and by direct action on striated muscle.

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THE ACTION OF HISTAMINE ON THE MOTILITY OF DIFFERENT PARTS OF THE INTESTINAL TRACT

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It has long been known that histamine exerts a powerful stimulating action on smooth muscle in different parts of the body, a single exception being found in the oesophagus of the turtle which is inhibited by this substance (1). Since it is possible that histamine may be formed in the intestine the question of its action on the smooth muscle of the alimentary tract becomes an important one. The majority of the investigators who studied this problem were concerned with the effect of this drug only on isolated strips of gut (2, 3, 4, 5, 6, 7, 8, 9). Although this method is of certain value in the analysis of the action of different substances on the intestinal muscles it gives practically no information concerning the action of a drug in the whole animal. It was therefore considered desirable to reinvestigate the action of histamine on the motility of the gut, by observing its effect on the intestine of the intact animal as well as on isolated strips.

In the experiments on the intact animal histamine was given either intravenously or subcutaneously or was injected into the lumen of the intestine, its subsequent effect being observed on different portions of the gut.

DESCRIPTION OF METHODS. Dogs and cats were used for the investigations in the whole animal. These animals received only milk the day before the experiment. In the majority of cases they were anesthetized with ether and a mixture of chloralose and urethane (0.1 gram of chloralose per kilogram of half the body weight and 1 gram of urethane per kgm. of half the body weight), injected intravenously through a cannula previously introduced into the femoral vein. In a few experiments chloralose 0.1 gram per kilo was given. Tracheotomy was then performed and in some cases a blood pressure cannula inserted into the carotid artery. A midline abdominal incision was made and in most experiments ligatures placed around the splanchnics and vagi. Three portions of gut were then separated as follows. A piece of duodenum about two inches long was measured off below the entrance of the pancreatic and common bile ducts. A longitudinal slit was made through the muscular layer at one end of the segment, and the mucous and submucous layers were separated with a blunt instrument from the muscular layer and tightly ligated. A cannula was then inserted into the end of the duodenal segment through a small incision in the mucous membrane. This cannula was tied in place by a second

ligature around the mucous membrane at the point where the latter was separated from the muscular layer. In this way one end of the duodenal segment was separated and a cannula inserted into its lumen without ligaturing the muscular layers or in any way interfering with the continuity of Auerbach's plexus or the blood supply. The other end of the duodenal segment was treated in exactly the manner described above, except that a T tube was introduced instead of a straight cannula. Loops of jejunum and ileum were also prepared like the duodenum with a cannula in each end. In this way three segments of gut were separated without the muscle layers being injured and without any ligatures being placed around the intestine. After the segments had been separated, each in turn was washed through with warm Ringer solution. The cannulae were then clamped off and the whole animal put in a bath of Ringer solution at 38°C. In this way the intestines were kept immersed constantly in a warm isotonic solution. In the experiments concerned with the action of histamine from the lumen of the gut an extra loop of intestine (duodenum or ileum) was prepared as just described for the injection of the drug.

The movements of the separated segments were recorded by the so-called "filling method" described by Babkin (10). The T cannula in the end of each segment was attached to a Marey capsule and the other opening closed by a clamp. Warm Ringer solution was then introduced in turn into each of the three segments through the straight cannulae in their proximal ends. During the filling of the loops the tubes leading to the Marey capsule were clamped off and the other part of the T tube opened. It was usually necessary to adjust the tension in the loops in order to obtain good contractions of the gut. Precautions were also taken to try to keep as near the same tension as possible in the three segments at any one time. The intestine was kept fully immersed in the bath by attaching Spencer Wells forceps to the mesentery, care being taken that the three isolated segments were at the same distance from the surface of the solution. In addition to recording the movements on a smoked paper, the intestines were observed visually to distinguish the type of contractions taking place.

The doses of histamine used for intravenous injection varied from $\frac{1}{4}$ to 2 mgm.; for subcutaneous administration doses of 1 mgm. were used, while amounts as large as 100 mgm. were introduced into lumen of the intestine.

The effect of intravenous injection of histamine on the denervated gut. In the beginning of the experiment the vagi were cut and splanchnics severed by quickly pulling the ligatures previously placed around them. In this way the intestine could be denervated without disturbing the position of the animal in the bath. As the greater part of each experiment was performed on the denervated gut, its reaction will be discussed first.

A. *Cats.* It was observed in all the experiments that after section of the nerves the motility of the intestine appreciably increased, as did also its response to histamine. A typical picture of the reaction of the duodenum, jejunum and ileum to intravenous injection of $\frac{1}{4}$ mgm. of this drug is seen in figure 1. As may be seen from this figure, different parts of the intestine react differently, the response being most marked in the ileum, less marked in the jejunum and least in the duodenum. In addition to there being a difference in the strength of the reaction, there is also a characteristic type of movement in each of the three parts of the gut.

This characteristic response to histamine was, with two exceptions, invariably observed in the experiments on cats.

The effect of intravenous administration of histamine on the different parts of the intestine may be divided into three phases. First, it will be noted that there is a very strong initial contraction in all three loops. This contraction usually appears in the duodenum and jejunum slightly before the ileum. It takes place rapidly and is very pronounced. Direct observation with the eye showed that it was due to an intense shortening of the segments of gut, this phenomenon being sometimes so marked that the intestine became quite blanched. No circular rings of contraction were observed in this initial stage. This contraction quickly relaxes and is followed by the second phase. In this phase there is a period of inhibition in which the intestine is relaxed following the first contraction. A marked vaso-dilatation is seen in this stage, the intestines being quite flushed. The period of inhibition is usually most pronounced and of longest duration in the duodenum. It is also well marked but of shorter duration in the ileum. Occasionally in the jejunum complete relaxation does not take place and the period of inhibition is not observed. This reaction is not shown in figure 1 but was seen in a number of experiments.

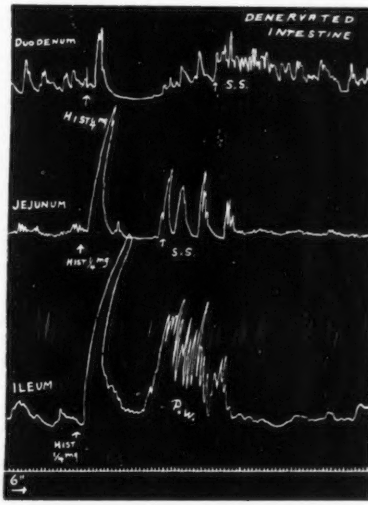

Fig. 1. Shows the typical reaction of the duodenum, jejunum and ileum to intravenous injection of histamine. *S. S.* refers to contractions produced by shortening and widening of the intestinal segment; *R. S.*, to contractions defined as rhythmic segmentation, and *P. W.*, to contractions of a type of peristalsis.

In the third phase the duodenum gradually returns to its normal rhythm sometimes with heightened tonus and increased contractions, which often consist of pulsating, rhythmic rings of contraction remaining in one place. According to the definition of Cannon such movements may be described by the terms rhythmic segmentation. In the jejunum the contractions in the third phase are chiefly due to shortening and widening of the segment of gut, although occasionally rhythmic segmentation may be observed. The third phase in the ileum is quite characteristic and differs from that seen in the other two parts of gut. This phase usually begins after the

period of relaxation and takes the form of a type of peristalsis, consisting of one or more rings of contraction which travel as waves along the ileum. Such waves of contractions were not observed in the duodenum or jejunum following histamine.

It is interesting to note that local application of a histamine solution containing $\frac{1}{4}$ mgm. in 1 cc., to the outside of the three segments of gut evoked a response similiar to that observed with intravenous injection of the drug. Thus the duodenum reacted the least with an initial shortening of the segment of gut, followed by few contractions which were chiefly due to further shortening and relaxation. In the jejunum the type of

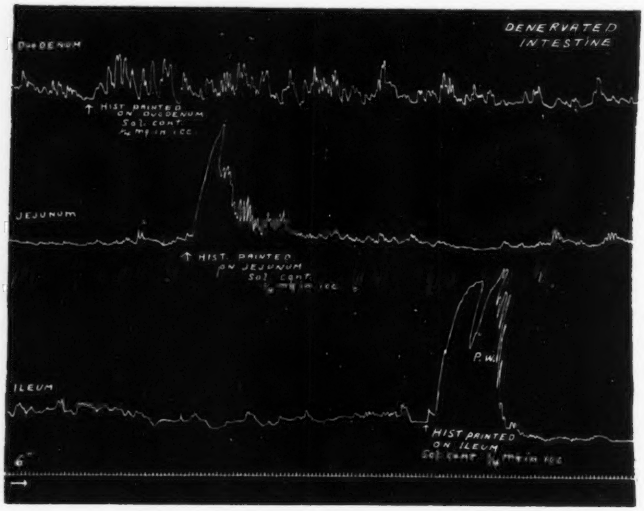

Fig. 2. Shows the typical reaction of the duodenum, jejunum and ileum to local application of histamine.

response was the same as in the duodenum but more marked. The ileum reacted most strongly to local application of histamine, giving first a shortening of the longitudinal layer which was followed by active movements of a type of peristalsis. An example of the reaction of the three segments of the intestine to local application of histamine is seen in figure 2.

Several experiments were performed with only two loops of gut, the upper part of the jejunum and the lower part of the ileum. In these experiments the blood pressure was recorded on the tracing simultaneously with the movements, as it was thought that the second phase of relaxation might be due to the fall of blood pressure. However, as this relaxation

was not observed until after the blood pressure was returning to the normal level, it does not appear as if the condition of the general circulation was directly responsible for it. Since small doses of histamine activate the secretion of adrenaline, this latter substance could be responsible for the relaxation of the gut and for the not infrequent slight secondary rise of blood pressure, which occurs after the primary fall produced by histamine. For an example of this type of experiment see figure 3.

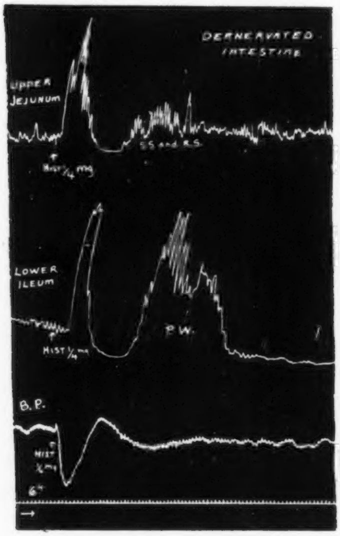

Fig. 3

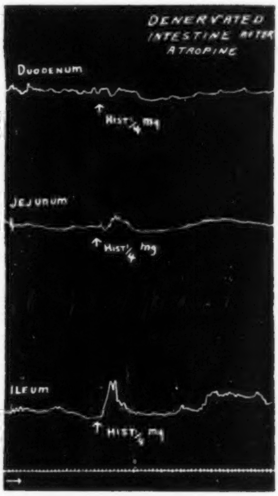

Fig. 4

Fig. 3. Shows the changes in blood pressure after histamine occurring simultaneously with the increased motility of the gut.

Fig. 4. Shows the response of the duodenum, jejunum and ileum to histamine after the administration of atropine.

B. *Dogs.* Somewhat similar results could be seen in dogs with denervated intestine. In these animals, as in cats, the activity was greater in the lower parts of the intestine than in the duodenum. All three segments responded with a three-phase curve, i.e., contraction, relaxation and series of contractions, although the period of relaxation was much longer than that observed in cats. However, in dogs the specificity of reaction in the ileum was not so marked as in cats, since travelling rings of contraction were sometimes observed in the jejunum and even in the duodenum following histamine.

The effect of intravenous injection of histamine on the gut retaining its

extrinsic innervation. The effect of intravenous injection of histamine on the intestine with the nerves intact is the same as its effect on the denervated gut but much weaker.

It has been noted in both the intact and denervated intestine that after repeated intravenous injections of histamine the second and third phases of its action tend to diminish and finally disappear. This is especially true of the travelling rings of contraction observed in the ileum.

The action of atropine on the movements of the gut stimulated by histamine. It has been found that intravenous injection of atropine in doses of 1 mgm greatly diminishes but does not abolish the action of $\frac{1}{4}$ mgm. of histamine. (See fig. 4 and compare with fig. 1 from the same experiment before the injection of atropine.) The effect of atropine is most marked on the duodenum, as it practically abolishes the action of histamine on this portion of the gut. In the jejunum the effect of the former drug is not quite so marked as in the duodenum, while in the ileum it is still less pronounced. With larger doses of atropine (4 to 5 mgm.), which were sufficient to abolish completely the action of acetyl choline, histamine in doses of 1 to 2 mgm. still gave a motor reaction. This reaction was weak and incomplete in the duodenum and jejunum but more marked in the ileum. In the latter portion of the gut a travelling ring of contraction was even observed with the above-mentioned dose of histamine after atropine. Thus it may be concluded that after paralysis of the parasympathetic nerve endings the ileum is most reactive to histamine.

It is interesting to note that similar results were obtained when histamine was applied locally to the intestine after atropine, i.e., its effect was most marked on the ileum, much less so on the jejunum and practically abolished in the duodenum.

The action of subcutaneous injection of histamine on the motility of the gut. It was reported by Ivy and Vloedmann (11) that histamine given subcutaneously had no effect on the movements of the stomach of dogs with gastric fistula. In accordance with their results we have found that subcutaneous injection of 1 mgm. of the drug in cats has no effect on the motility of any of the three segments of isolated gut. Larger doses of histamine than 1 mgm. have not been tried.

The action of histamine introduced into the lumen of the intestine on the movements of the gut. There are divergent opinions expressed in the literature concerning the absorption of histamine from the small intestine. Meakins and Harington (12) found that in cats, following the injection of histamine into an intestinal loop, a sudden marked fall in blood pressure occurred. On the other hand Wangensteen and Loucks (13) similiarly using the fall in blood pressure as an index of absorption were unable to confirm the results of Meakins and Harington on dogs.

In the present investigation histamine solutions containing in some cases

doses as large as 100 mgm. were introduced into the intestine of cats. Different loops of the gut were used for this purpose, in some cases the ileum and in other cases the duodenum and jejunum. The portion of the gut chosen was about 10 cm. long and was separated off, as described above in the methods, without injuring the blood supply or the muscle coats. Blood pressure and the movements of two additional loops of gut were taken as an index of absorption of the drug.

In contrast to the findings of Meakins and Harington there is from these experiments very little evidence that the histamine introduced into the lumen of the gut is absorbed in amounts sufficient to influence greatly either the motility or the blood pressure within a period of one-half to two hours. In several experiments, to be sure, there was a very slight gradual fall in blood pressure, which may have been due to the absorption of very minute amounts of the drug. However, even in these cases no appreciable change in intestinal motility was observed. A possible explanation for the very different finding of Meakins and Harington is that a different anesthetic, namely, paraldehyde was used in their experiments.

The question also arose as to whether the histamine was destroyed in the lumen of the intestine. Mellanby (14) found that this substance disappeared quite rapidly from the intestine provided the circulation was not interfered with. Koessler and Hanke (15) also found that when histamine was fed by mouth to dogs and guinea pigs, large amounts disappeared while in the gut although there was no evidence for its absorption. The recent findings of Best (16) who has found a histamine inactivating enzyme in the intestinal mucosa, may explain the disappearance of this substance when introduced into the intestine. On the other hand, under the conditions of my experiment there is no conclusive evidence for the destruction of large amounts of histamine during the time it is present in the gut. This is evinced by the fact that when the histamine solution is removed after one to two hours in the intestine, made up to the original volume and diluted for intravenous injection, it causes a fall in blood pressure of the same magnitude as that produced by some of the original solution similiarly diluted and which had not been placed in the gut.

An interesting observation which may point to an action of histamine from the intestine was made in an experiment on a dog. This animal had refused food and had been suffering from diarrhea before the experiment. Upon intravenous injection of histamine it was noted that its usual action on the motility of the gut was reversed, that is, it produced the greatest activity in the duodenum, less in the jejunum and least in the ileum. In this case it was observed that there was a profuse secretion of yellowish fluid into all parts of the gut, so that all the intestines were distended. Several hundred cubic centimeters of this fluid were drawn out from the gut by means of a hypodermic syringe. The isolated segments were then

washed through several times with Ringer solution and refilled. After this procedure the intravenous injection of histamine was followed by its usual effect, the contractions being most marked in the ileum and less in the upper portions of the gut.

The action of histamine on isolated pieces of gut taken from different portions of the small intestine. Since the response to histamine was found to vary quite markedly in different portions of the intact small intestine, some experiments on the effect of this substance on different pieces of isolated gut were tried. These pieces were taken from the same regions of the small intestine which had been investigated in the intact animal.

Cats were used for these experiments. They were first anesthetized with ether and chloralose, the abdomen was then opened and pieces of the

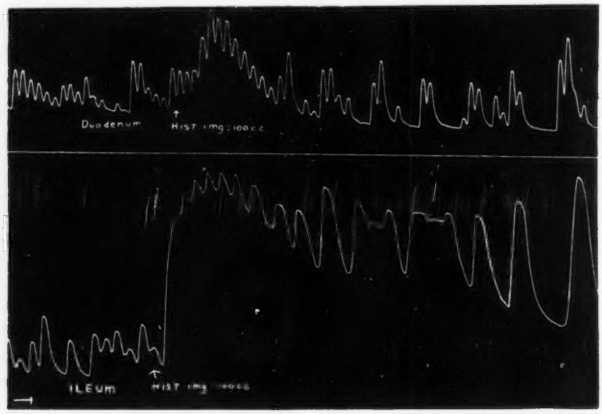

Fig. 5. Shows the response of isolated strips of gut to histamine

intestine removed from the duodenum, jejunum and ileum. The length of these pieces was 1 to 2 cm. long; they were washed with warm Ringer solution, immersed in a bath of oxygenated Ringer at 38°C. and attached at either end by a bent pin. The pin at the lower end was fixed while the upper was attached to a thread and writing lever. In this way contractions of the longitudinal layer were measured. Histamine in amounts of 1 mgm. were added to the 100 cc. of Ringer solution in which the strip of gut was immersed.

In the isolated gut as in the intact intestine it was found that the response to histamine was much more marked in the ileum than in the duodenum or jejunum. In the duodenum in some cases a rise in tonus was observed following histamine, the number of contractions being diminished

but their force increased. The reaction was similiar in the jejunum but usually not as well marked as in duodenum. Indeed in a number of cases histamine did not affect or had only a slight action on these two portions of gut. On the other hand the ileum invariably responded to histamine. A very marked rise in tonus took place much greater than that observed in other portions of the gut. As this increased tonus gradually relaxed the rate of the contractions decreased but their force was very much augmented (fig. 5).

The effect of atropine on the movements of the gut produced by histamine was tried. This substance in doses of $\frac{1}{2}$ mgm. in 100 cc. while it diminished slightly the spontaneous rhyhtm of the isolated strips did not affect the action of histamine.

Conclusion. It may be concluded that histamine introduced intravenously provokes a typical motor reaction in different parts of the small intestine. This reaction is specific regarding its strength, course and character. Local application of the drug to the different parts of the intestine also evokes a characteristic response similar to that produced by intravenous administration. The same typical motor reaction was observed on isolated strips taken from different parts of the duodenum and of the small intestine.

The usual effect of histamine, i.e., more marked reaction in the ileum and less marked in jejunum and duodenum, was observed as a rule. There were very few exceptions, one of the most typical of these being the case of the dog suffering from diarrhea, in which the normal response of the intestine to histamine was reversed. No analysis of the causes of the specific reaction of different parts of the intestine to histamine has been performed in this investigation. But histamine, as many other substances, especially those in the class of "Natural Chemical Stimuli" (Babkin, 17; Ducceschi, 18; Goldenberg 19, and others), is endowed with the property, to produce a typical reaction under certain conditions.

SUMMARY

1. Histamine when injected intravenously in doses of $\frac{1}{4}$ to $\frac{1}{2}$ mgm. in the intact animal activates a quite characteristic motor response in different parts of the intestine. Its effect is most marked in the ileum and decreases towards the duodenum.

2. Local application of a histamine solution to the outside of the gut also evokes the typical reactions in different portions of the intestine which are seen after intravenous injection of the drug.

3. After paralysis of the parasympathetic nervous system by atropine the characteristic action of histamine on the different parts of the intestine is partly diminished but not abolished. This inhibitory effect is most marked in the duodenum and least in the ileum.

4. Subcutaneous injection of 1 mgm. of histamine produced no change in the normal rhythm of the intestine.

5. Injection of a solution of histamine containing amounts as large as 50 mgm. of the drug into the lumen of different parts of the intestine did not activate a motor response.

6. Histamine in concentration of 1/100,000 has a greater stimulating action on isolated strips of ileum than on isolated strips of jejunum or duodenum. Atropine does not abolish this effect.

I wish to express my thanks to Dr. B. P. Babkin for very helpful advice received throughout the course of this work. I also acknowledge my indebtedness to Dr. R. L. Stehle for the use of facilities of his laboratory in carrying out the experiments on the isolated gut.

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STUDIES IN THE PHYSIOLOGY OF VITAMINS

XIV. The Effect of Administration of Large Amounts of Water on the Time Required for Development of the Anorexia Characteristic of a Deficiency of the Vitamin B Complex

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One of the hypotheses concerning the cause of the neuritic symptoms of vitamin B deficiency attributes these manifestations to the presence of a toxin. The startling cures that occur following vitamin administration to so-called polyneuritic pigeons, in which nerve degeneration can be demonstrated so readily, can hardly be explained as due to sudden correction of the degenerative changes in nerves. This has led many investigators to favor the toxemia idea. It has been suggested that the toxins may be either ingested with the food, developed from the materials in the alimentary tract, or be the result of a deranged tissue metabolism.

In their work with parathyroidectomized dogs Luckhardt and Rosenbloom (1921) found that they were able to prevent tetany by intravenous injections of large amounts of calcium-free Ringer's solution. The inference was that symptoms of parathyroid tetany may be due to a toxemia which can be prevented by production of a vigorous diuresis and washing out of toxins as fast as they are formed. This suggests a way of testing the toxemia hypothesis concerning the cause of the neuromuscular symptoms of experimental B-deficiency. If, in the presence of a maintained vigorous diuresis, animals should be able to subsist on a diet free from or very low in content of the antineuritic vitamin for a relatively long period, the finding would be of great significance and strongly support the toxemia idea. The only experimental work bearing directly on this question that we have been able to find recorded in the literature, is that of Eijkman and Van Hoogenhuyze (1916) who drenched birds from which food was withheld and observed that neuritic symptoms developed in from 19 to 30 days. These investigators concluded that their results did not support the toxin theory.

[1] This paper was prepared from the thesis submitted jointly by H. A. Rosenberg and J. Rogoff to the faculty of the Yale School of Medicine, June, 1930, in partial fulfillment of the requirements for the degree of Doctor of Medicine.

Evidence is at hand indicating that the state of hydration of the organism is not without influence on the syndrome of vitamin B deficiency. Work done on dogs (Stucky and Rose, 1929) and rats (Sure, Kik and Walker, 1929; Rose, Stucky and Mendel, 1930) indicates that anhydremia develops in advanced B-avitaminosis. Some pigeons, exhibiting neuromuscular manifestations suggestive of vitamin B deficiency, may be "cured" by administration of water alone, according to the observations of Jansen and Donath (1927) and Peters (1924). McCarrison, Sundararajan and Gloster (1928) observed varying degrees of edema in their birds maintained on diets deficient in the B factor.

One of us (Cowgill) has shown that dogs, subsisting on diets of which the casein III is representative, and having previously received large amounts of material rich in vitamin B, develop the anorexia characteristic of lack of this vitamin in about three weeks. Is it possible that, by forcing fluids to such animals, the period required for the development of this anorexia will be significantly diminished or lengthened? It was felt that the answer to this query might have some bearing on the validity of the toxemia theory referred to above. The following experiments were designed to yield data helpful in answering this question.

Experimental details. Five dogs, one as a control, were used in these experiments. They were fed the artificial food mixture *casein III*, details of which have already been published (Cowgill, 1923a). The general scheme of feeding was that commonly employed in this laboratory and described elsewhere (Cowgill, 1923b). As a preliminary procedure all of the animals received large amounts of vitamin B in the form of vitavose[2] in order that the amount of the vitamin "stored" in each dog might approximate the maximum possible. During a preliminary period of one week tests were made to determine the amount of water that each dog could tolerate by stomach tube at one administration without vomiting. The period of vitamin deficiency was then entered upon: each animal except the control was given daily, in addition to the usual allotment of vitamin-deficient food and water, as large a volume of water by stomach tube as could be tolerated without vomiting as often as circumstances allowed the experimenters to give it. Each dog was kept in a metabolism cage in order that the urine voided daily might be collected and its volume determined. The data on urinary output gave some indication as to the degree of diuresis being produced. When an animal developed anorexia no change in the procedure was made for about a week. The loss of appetite for the food offered was then proved to be due to lack of vitamin B, first, by negative tests with non-vitamin-containing materials such as sodium chloride and commercial beef extract, and second, by a positive

[2] From E. R. Squibb & Sons, New York, N. Y.

result with vitavose. For the week following, the original regime without vitavose was adhered to, water being forced as before. Following this phase of the study the animals were given a period of rest during which they received less expensive foods such as meat and dog biscuit supplemented by vitavose and water *ad lib.* When it was believed that each animal was in a state of hydration approximating that characterizing it at the very beginning of the study, the experiment was repeated *but without the forced administration of fluids,* until the anorexia again developed. After an appreciable period, during which some degree of appetite failure prevailed, the dogs were again given vitavose with subsequent restoration of the urge to eat.

RESULTS. *In every case of forced fluid administrction, the anorexia characteristic of lack of undifferentiated vitamin B developed in approximately one-half the time required when water was not so administered.* The control animal, subjected to the same experimental conditions but supplied with

TABLE 1

DOG	NUMBER OF DAYS REQUIRED FOR DEVELOPMENT OF ANOREXIA IN DOGS SUBSISTING ON DIET DEFICIENT IN UNDIFFERENTIATED VITAMIN B	
	With forced fluids	Without forced fluids
B	8	19
C	10	22
D	15	33
E	11	20
A (control)	not developed by 25th day	21

the vitamin maintained a perfect appetite for twenty-five days at which time the experiment was terminated. That this was a long enough test period for this animal is shown by the fact that, when deprived of the vitamin and not given large amounts of fluid, anorexia developed in twenty-one days, approximately the same time as was the case with the other animals of the group. The data are summarized in table 1.

It is of interest to point out that dog D, which required the longest period to develop anorexia when fluids were forced, also required more time than the other animals when fluids were not forced. Therefore, its behavior cannot be regarded as exceptional. It is possible that this animal had better utilization of the vitamin in its alimentary tract, or perhaps a slower excretion of this dietary essential. The importance of variability of gastro-enteric function in this connection has been emphasized by Minot (1929) as a possible cause of the variations that clinical cases exhibit.

DISCUSSION. From the above results it is clear that the onset of a loss of appetite in dogs on a diet lacking undifferentiated vitamin B may be

hastened by forcing fluids by mouth. The daily urinary output for each animal indicated very clearly that a pronounced diuresis was secured. There can be no doubt that the anorexia which developed was that truly characteristic of a lack of undifferentiated vitamin B. The possibility that it was occasioned by a loss of chloride associated with hydremic plethora (Rowntree, 1922) need hardly be entertained for several reasons: the amount of water given was insufficient; the diet contained liberal amounts of chloride; and, lastly, in every case, two doses of physiological saline were administered by mouth without effect on the anorexia. The administration of Liebig's commercial beef extract was likewise without effect thus confirming previous work reported from this laboratory. *In every case, when the vitamin* in the form of vitavose *was administered, however, there was the usual prompt restoration of the urge to eat.*

The hypothesis that the symptoms of lack of vitamin B are due to an accumulation of an abnormal toxic intermediary metabolite receives little support from these experiments. The results suggest that the voluntary restriction of fluid intake incident to a vitamin B-deficient régime is in the nature of a protective reaction. Perhaps the simplest explanation that might be offered is that elimination of the excess fluid that was administered resulted in washing out of the vitamin from the organism. In this connection the results of Van der Walle (1922) are of interest. This investigator noticed that the urine of a dog on a diet free from the antineuritic vitamin had no curative value for so-called polyneuritic pigeons, whereas the urine collected when the animal was subsisting on a normal diet was effective. Curatolo (1920) and Gaglio (1919) have also reported the presence of this vitamin in urine.

The results of this experiment may have some application in the field of clinical medicine. For example, the anorexia and the anhydremia observed in certain marasmic infants may be due, in part at least, to the insufficiency in amount of vitamin B which these infants receive. (Eddy and Roper, 1917; Daniels, Byfield and Loughlin, 1919; Hoobler 1928; Dennett, 1929.) One phase of the treatment which these infants often receive, namely, subcutaneous, intravenous and rectal administration of fluids, may actually be detrimental in that it washes vitamin B from an already partially depleted body. It seems logical, in the light of the experiments here reported, to combine the recognized and approved methods of treatment of such cases of marasmus with the administration of some potent source of vitamin B. Certainly sufficient evidence is at hand suggesting that trials of such therapy are warranted.

SUMMARY—CONCLUSION

Experiments on five dogs, one of which was a control, indicate that the administration of large amounts of fluid by mouth to an animal subsisting

on a diet relatively free from undifferentiated vitamin B markedly shortens the time required for the appearance of the anorexia characteristic of a lack of this dietary essential. This result is not in harmony with the hypothesis that the symptoms of B deficiency are essentially those of a toxemia. A practical application of these results is suggested in the clinical treatment of the marasmus in infants due to lack of vitamin B by administration of fluid *together with* the missing vitamin.

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THE RÔLE PLAYED BY THE VENTRICULAR RELAXATION PROCESS IN FILLING THE VENTRICLE

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At one time it was believed that the relaxing ventricle sucked blood into its chamber by some sort of activity during relaxation, such as an elastic recoil, a sudden stretching resulting from the filling of the coronary arteries, a marked asynchronous cessation of contraction, or even an actual contraction of some fibers at this time (cf. Ebstein, 1904; Tigerstedt, 1921, and Prince, 1915, for literature). The evidence presented in favor of this view has been refuted or, at least, shown to be compatible with the current idea of passive filling. For example, the recording of an intraventricular pressure below atmospheric with the minimal manometer and with the early graphic methods, by Goltz and Gaule (1878), de Jager (1883), Rolleston (1887), von Frey and Krehl (1890) and Porter (1892) among others, has been shown to be an instrumental error by Von den Velden (1906), Straub (1912) and Wiggers (1928). The latter two have failed to obtain pressures within the ventricle below that surrounding the heart when employing instruments of high precision constructed along the lines laid down by O. Frank (1903). Similarly, the claim of Mosso and Pagliani (cf. Ebstein, 1904) and of Stefani (1893) that the heart can fill when the pressure surrounding it is higher than in its chamber has been refuted by Roy (1879), Tunnicliffe (1896) and Martin and Donaldson (cf. Ebstein, 1904). The observation that the dead heart will fill after it has been artificially compressed (Chassaignac and Fick) (cf. Ebstein, 1904), does not necessarily apply to the conditions occurring in the living active heart (Von den Velden, 1906). The notch on the volume curve during early diastole to which diverse significance has been attached by Straub (1910) and de Heer (1912) is an instrumental artefact (Wiggers and Katz, 1920). The presence of a pressure gradient between the auricle and ventricle during the entire diastole with maxima early in the period and during auricular contraction (Porter, 1892) is equally compatible with the view of passive filling as with that of suction (Henderson, 1906).

Evidently the proof of a suction action by the relaxing ventricle is lacking. The current view of ventricular filling conceives the ventricles as being entirely passive, i.e., that the ventricular walls are stretched by

the force of the pressure head in the veins and auricles and that the energy of filling comes entirely from this pressure head, which is created by the accumulation of blood returning to the veins and auricles.

The idea that the ventricle plays no rôle in filling ignores the fact that the ventricle, like ordinary skeletal muscle, is an elastic body which has one elastic state when contracted and another when relaxed (Frank, 1895; Gasser and Hill, 1924). The process of contraction can be viewed as a change from the relaxed to the contracted elastic state and the process of relaxation as the reverse. Wiggers (1927) has shown that if the ventricle is not fully relaxed when filling begins, the completion of relaxation will facilitate filling. In fact, if the ventricle were to stay fully contracted as occurs with certain poisons, the amount of filling which the accumulation of fluid in the veins might produce is obviously much smaller than when relaxation occurs. A priori, these considerations indicate that the relaxing ventricle is not entirely passive but plays some rôle in filling.

The intact mammal is not very suitable for evaluating the rôle of ventricular relaxation in filling. This evaluation can be more readily accomplished in an isolated system where the other variable factors can be kept constant and relaxation compared with contraction. In this system a filling reservoir should be employed of such large cross section area that the removal of fluid from it to fill the heart will not appreciably alter the fluid level in it. The reservoir will keep the pressure in the system and against the walls of the ventricle constant unless some change is induced by activity of the heart. The use of a heart such as the turtle's with the auricles removed will further simplify matters by reducing the active chambers to one. Similarly, the use of rigid glass tubes to connect the ventricle with the filling chamber will eliminate the variable effect of elastic tubes. A narrow constriction in the tubes through which the ventricle fills will exaggerate any pressure variations which activity of the ventricle might produce and so make it more appreciable. The onset of filling may be made to coincide closely with the beginning of relaxation, in order to get the full effect of relaxation, by making the ventricle fill from the same reservoir chamber that it empties into. This arrangement will also make the extracardiac conditions during filling and emptying practically identical and thus allow a better comparison of the two phases. With the above arrangement contraction and relaxation would occur under conditions which approached an isotonic state. An investigation of the rôle of relaxation in filling was undertaken along these lines.

Method. A set-up modified from that previously described (Katz, 1928) was employed to satisfy the above mentioned conditions. A diagrammatic representation of the arrangement is given in figure 1. The turtle ventricle was connected with a reservoir, *E*, containing isotonic sodium chloride, by means of a glass tube, *N*, and cannula, *I*, constricted

at the end. This cannula was inserted into the ventricular chamber via the aorta. In these experiments the stopcock, *A*, was turned 180° from the positon shown in the diagram to exclude the tubes *G* and *H*. The saline reservoir was of such large cross section area that the fluid level dropped only 1 mm. for each 6.5 cc. saline removed. Intraventricular pressure was recorded by a modified Wiggers manometer, *P*, inserted into the ventricular cavity via the A-V orifice. Ventricular volume was recorded from an air-filled chamber, *J*, connected with a Frank segment capsule, *Q*, by a rubber tube, *F*. During the course of an experiment stopcock *C* was turned 90° and the side tube *B* was completely closed off. The pressure and volume changes were simultaneously recorded on bromide paper without parallax by using a double beam of light from the author's (1924) duo-slit lamp. Such records were obtained at various levels of the reservoir. The volume curve was calibrated by means of the graduated pipette *L*, and the pressure curve, by varying the height of the reservoir, *E*, after the heart had ceased to beat.

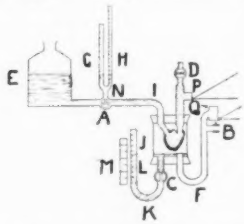

Fig. 1. Diagrammatic representation of set-up for isolated turtle ventricle. *A*, stopcock (turned 180° from position shown in these experiments); *B*, side tube (closed in these experiments); *C*, stopcock (closed in these experiments); *D*, stopcock; *E*, saline reservoir; *F*, rubber tube; *G* and *H*, tubes (not used in these experiments); *I*, glass cannula inserted into ventricle, end of this cannula constricted; *J*, air chamber plethysmograph; *K*, rubber tube; *L*, graduated tube for calibrating volume curve; *M*, disregard; *N*, glass tubing connecting reservoir with cannula *I*; *P*, modified Wiggers' manometer for recording pressure of ventricular chamber; *Q*, Frank segment capsule for recording volume of ventricle.

Results. The records obtained demonstrate without fail that the pressure early during diastole falls below the zero level of the system. A typical record is shown in figure 2. During ejection of fluid, which is indicated by the decrease in the volume curve, *V*, the pressure, *P*, rises for a time and then falls, almost reaching the zero level of the system by the end of ejection (indicated by the horizontal line in fig. 2). During filling which is indicated by the rise of the volume curve the pressure falls below the zero level at first and then returns to it. The reduction in pressure during filling is almost equal to the elevation of pressure during ejection in this particular experiment.

A reduction of pressure below the zero level of the system occurred in all the preparations during filling as long as the ventricle was rhythmically active. The reduction in pressure below the zero level was found to be greatest when the zero level of the system was highest[1] and when the

[1] In these experiments, the optimum filling pressure was not reached.

ventricular activity was most vigorous. The amount of pressure reduction during relaxation varied more than the amount of pressure elevation during contraction as the reservoir level was varied. At no time was a pressure below atmospheric recorded within the ventricle.

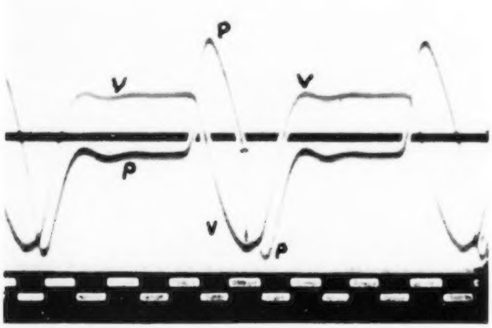

Fig. 2. Simultaneous pressure, *P*, and volume, *V*, curves obtained under nearly isotonic conditions. Horizontal black line is base line of pressure curve. At bottom, white line is time; each change in level represents one second.

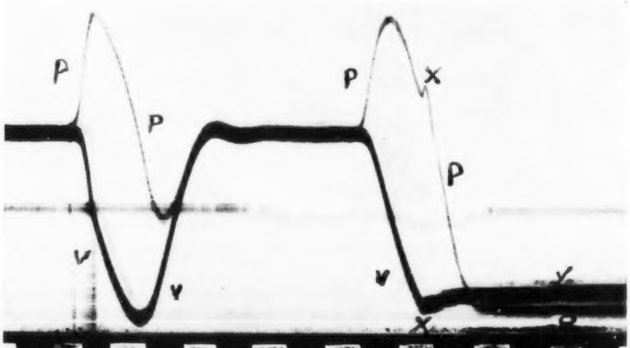

Fig. 3. Simultaneous pressure, *P*, and volume, *V*, curves obtained under nearly isotonic conditions and under isometric conditions. Points *X* indicate time when conditions changed from isotonic to isometric. Base line and time as in figure 2.

The objection may be raised that the reduction in pressure during relaxation is an artefact caused by an overswing of the system and is not produced by the relaxing ventricle. The gradient of the change is too steep and the pressure drop is of too long duration to be explained as a

free oscillation of the system; it is rather like a forced phenomenon. Furthermore, the variation in pressure and volume at first go in opposite directions unlike the true free oscillations of the system sometimes recorded after filling is over (cf. fig. 2) where the pressure and volume changes are parallel and in the same direction. However, further proof was devised to show that the pressure reduction was not an artefact but actually evidence of activity on the part of the relaxing ventricle. It was based on the idea that the filling of the ventricle with fluid tended to decrease the

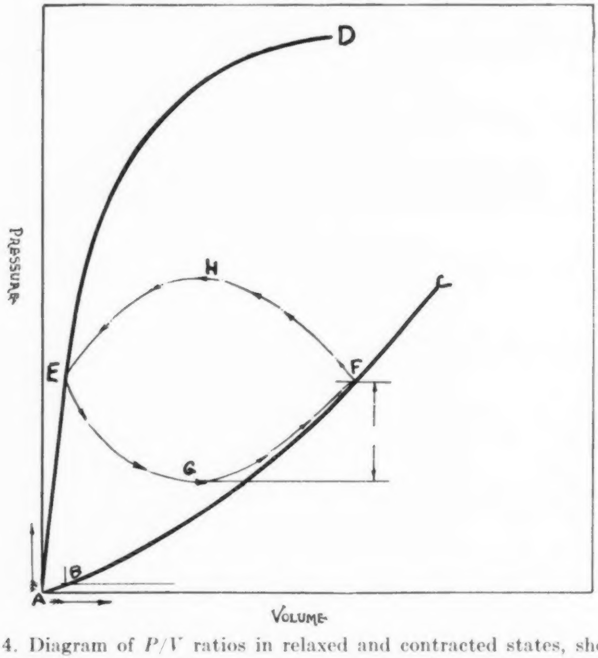

Fig. 4. Diagram of P/V ratios in relaxed and contracted states, showing diagrammatically changes during contraction and relaxation. Discussed fully in text.

amount and abbreviate the period of pressure reduction. On this basis, a greater reduction in pressure would be anticipated if ventricular filling were prevented. Filling could be readily prevented with the apparatus employed by turning stopcock A (fig. 1) 90° at or near the end of the ejection period, thereby cutting off the ventricle from the reservoir. This procedure was carried out a number of times and, without fail, it was found that the pressure drop was magnified. A typical record is shown in figure 3. The first beat of the record shows the volume, V, and pressure, P, changes when the ventricle was allowed to fill. The changes are essentially

identical with those already described and illustrated by figure 2. At X in figure 3, just before the end of ejection, stopcock A was turned and the ventricle separated from the reservoir. The ventricle, therefore, did not fill as shown by the horizontal level maintained by the volume curve, V, and the pressure, P, rapidly dropped more than double the distance below the zero level found when the ventricle did fill.

This experiment is similar to that of making the ventricle contract isometrically where, as is well known, the pressure rises higher than under nearly isotonic conditions. The relaxing ventricle therefore behaves qualitatively in a manner similar to that of the contracting ventricle when the conditions are changed from nearly isotonic to isometric conditions. The reason for the increase in the pressure change when the relaxation (or contraction) of the ventricle occurs isometrically over that under nearly isotonic conditions, lies in the change in elastic state of the heart muscle during the process of relaxation (or contraction). During relaxation the heart, like skeletal muscle, changes from a body with a high elasticity coefficient to one with a low elasticity coefficient; i.e., the ratio P/V decreases (where P is the pressure and V is the volume). During contraction the changes are in the reverse direction (cf. Frank, 1895; Gasser and Hill, 1924), i.e., the ratio $\frac{P}{V}$ is increased. A similar change can be produced in the ratio $\frac{P}{V}$ by changing the numerater, P, by altering the denominator, V, or by doing both. If the ventricle can fill during relaxation, the ratio $\frac{P}{V}$ is decreased by an increase in the volume, V, and a decrease in the pressure, P; but when isometric conditions prevail the change in P/V is accomplished by decreasing P alone. Hence, the change in P is larger under isometric conditions than when filling can occur.

These facts can be illustrated in the diagram of figure 4. The upper curve AED represents the $\frac{P}{V}$ ratios of the fully contracted ventricle (cf. Frank, 1895) and the lower curve AFC, the ratios of the fully relaxed ventricle. Now, if we start with the fully relaxed ventricle at point F, then the changes occurring during contraction when the ventricle is connected with the reservoir (as in fig. 1) would fall along a curve such as the dotted line FHE (fig. 4), the temporary rise of pressure being due to the friction in the tubes and the inertia of the fluid retarding the ejection of fluid. During relaxation, if filling occurs, the changes will fall along a curve such as the dotted line EGF; the temporary fall in pressure being due to the friction in the tubes and the inertia of the system (possibly plus the initial momentum in the opposite direction) causing a lag in the movement of fluid toward the heart. The degree to which the pressure will fall during filling

will depend on the relation of the length and calibre of the tubes to the rate of relaxation. The shorter and wider the tubes or the slower the change in elastic state, the less marked will be the pressure drop. The faster the change in elastic state and the longer and narrower the tubes, the more marked will be the pressure drop. When filling is prevented, making the relaxation isometric, the pressure drop will be at a maximum, i.e., the vertical height *EB*. In other words, the drop in pressure during

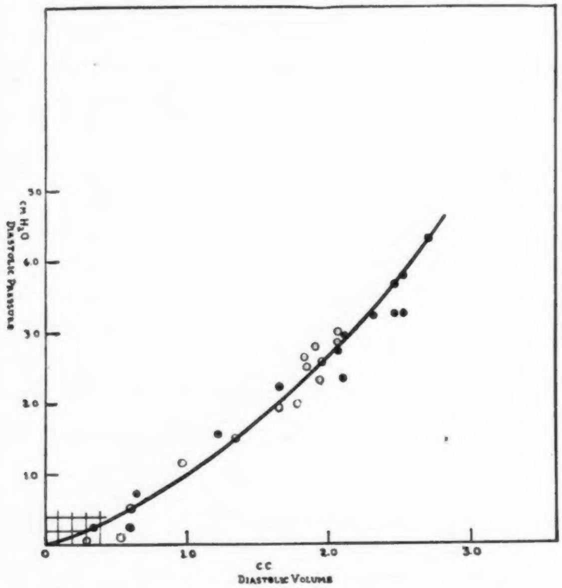

Fig. 5. A comparison of the P/V ratios of the fully relaxed ventricle, relaxing nearly isotonically (closed circles) and relaxing isometrically (open circles), with the smooth curved line.

relaxation is due to the inability of the volume changes to keep pace with the change in the ratio $\frac{P}{V}$, and the extent of the pressure drop is a direct function of this lag in flow of fluid toward the ventricle.

Although the pressure changes during relaxation can be varied by changing the degree of filling, by altering the rate of filling, or by preventing filling entirely, nevertheless the curve representing the $\frac{P}{V}$ ratios of the fully relaxed ventricle is not affected by any of these factors. It was found in these experiments (cf. fig. 5) that in any single heart the

P/V ratios of the fully relaxed ventricle coincided reasonably with the same smooth curve both when the volume was set and relaxation was isometric (cf. open circles, fig. 5) and when the pressure was set and relaxation nearly isotonic (cf. closed circles, fig. 5).

Discussion. The experiments cited in this report indicate that, under the conditions of these experiments, filling occurs as a result of the relaxation process which reduces the elastic coefficient P/V to a minimum value. By so doing, the pressure within the ventricle drops and so establishes a pressure gradient which tends to cause a movement of fluid toward the heart.

Several questions remain to be discussed: 1. What are the sources of energy for the change in elastic state? 2. Do these experiments indicate that the relaxing ventricle aspirates blood? 3. What is the relation of the results of these experiments to the common observations on the isolated muscle suspended on mercury? 4. Does the relaxing ventricle play a similar rôle in filling in the intact animal?

On an energetic basis, the fact that the pressure in the ventricle is reduced below the zero level of the system indicates that some work has been done and some source of potential energy has been converted to free energy. The ultimate source of such energy is some potential store created by the contraction process. It may come in part from the exothermic chemical reaction, presumably a neutralization of acids similar to that which Hill, Meyerhoff and their collaborators have shown occurs during the relaxation of skeletal muscle. The store of mechanical energy, built up during contraction, which would be converted to free energy might be in several forms. During contraction the potential energy of the heart muscle would increase because the muscle changes from a body with a low to one with a high elasticity coefficient. It would also increase because the center of gravity of the heart would be raised in the system used in these experiments. There might be some energy stored in the strain of some of the non-contractile elements in the heart. During relaxation these sources of energy and possibly others would be set free and could do work, at least sufficient to tend to drop the pressure within the ventricle below the zero level of the system. The reason that the pressure does not go below atmospheric is of course due to the limit set to the conversion of potential to free energy. The heart has a minimum elastic state which allows the pressure to approach atmospheric zero but never to fall below it. In other words, the lower limit of the pressure is set at atmospheric pressure by the minimum elastic state of the heart.

While the energy which enlarges the heart and lowers the pressure within its chamber is created by the heart, the energy for fluid movement comes from the pressure head in the reservoir. The filling heart in this regard differs in no way from any suction or aspirating pump in which

some source of energy lowers the pressure in the pump, but the energy for flow comes from the pressure head in the reservoir regardless of what this level is in relation to atmospheric pressure. The only apparent difference between the ventricle and other aspirating pumps is the fact that the heart cannot lower the pressure within its chamber below that surrounding it. Nevertheless it can draw fluid into its chamber. Under these experimental conditions, therefore, ventricular filling is a process similar in nature to emptying. The mechanism for both filling and emptying is dependent

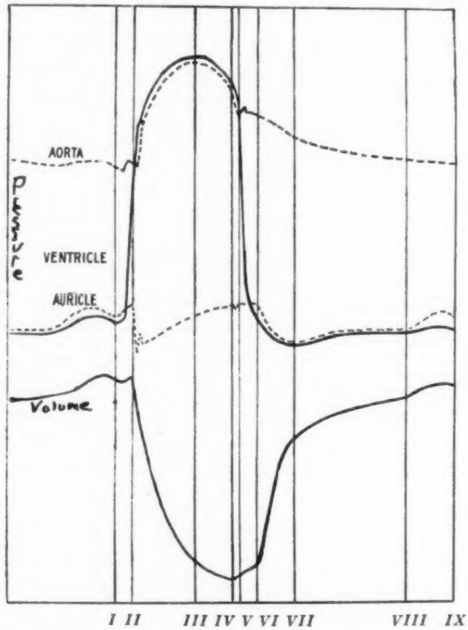

Fig. 6. Pressure curves and volume curve of the mammalian heart (after Wiggers), discussed in text.

on some reversible physico-chemical process which alters the elastic coefficient of the ventricle between two definite limits, the maximum for the contracted and the minimum for the relaxed ventricle.

The observations here reported are readily correlated with the experiments on skeletal muscle placed on mercury. It has been reported that when a muscle is placed on mercury and stimulated, it will shorten and not lengthen again. As a matter of fact, at first there is a slight lengthening and eventually as the resting length decreases, a state is reached where there is no shortening either. There is no doubt that the absence of

lengthening and later of shortening is due to the inability of the reduced change in elastic state, which decreases as the resting length decreases, to overcome the friction and other resistances preventing change in length. A few observations bearing on this point have been made by the author on the isolated frog gastrocnemius connected with a balanced lever and so arranged that lengthening will occur against gravity. It was found that during relaxation the gastrocnemius even with a small initial length can push the lever and so must do a little work. The amount of this work, although much less than that done in contraction, is nevertheless quite noticeable.

Granting then that the isolated ventricle connected to a reservoir, as in these experiments, can exert a sucking action and draw fluid into its chamber, the question is whether or not the mammalian ventricle actually does draw blood into its chamber when the circulatory system is intact. Evidence on this question can be obtained from an analysis of the combined ventricular volume and pressure curves of the dog's heart such as Wiggers prepared (cf. fig. 6).

Before discussing the curve, it should be borne in mind, that beside the possible sucking action of the ventricle two other factors are concerned in filling the ventricle, namely, 1, auricular activity, and 2, elevation of the pressure head in the auricles and veins by blood returning to them. During filling, the changes are different in the various phases. During rapid inflow (VI to VII) the pressure drops and the volume increases; during diastasis (VII to VIII) and early auricular activity, both pressure and volume increase, only to decrease somewhat later in auricular activity. The fact that the pressure decreases during rapid inflow while the ventricle is filling, indicates that the ventricle is relaxing since the ratio $\frac{P}{V}$ is decreasing. Were filling entirely due to a passive distention, during this phase, one would expect to find an increase in both the volume and pressure as is the case during distention of any elastic body and is actually the case during diastasis and early auricular activity. The fact that the pressure drops during filling indicates that the ventricle is relaxing at a faster rate than it can fill. Such a state of affairs is inconceivable if the filling were entirely passive during this phase. In other words, since the large and rapid increase in volume in the rapid inflow phase is accompanied by a decided drop in pressure, the inference is obvious that a decrease in the elasticity coefficient is primarily responsible for the pressure drop and for filling. In no other way can one rationally explain, it seems to me, the opposite changes in volume and pressure. The relaxing ventricle, therefore, not only can but does exert a sucking action to draw blood into its chamber. The relative importance played by this sucking action, by auricular activity, and by passive filling due to the accumulation of

blood returning to the heart, remains to be worked out under various conditions for the two ventricles.[2]

SUMMARY

1. Experiments were made on the isolated turtle ventricle connected to a reservoir, which tended to keep the pressure of the system constant, in order to see what rôle ventricular relaxation played in filling the heart chamber.

2. It was found that during relaxation the pressure within the ventricle dropped below the zero level of the system. The amount of this drop was a direct function of the initial length of the muscle in the range studied.

3. The drop in pressure was not due to an overswing, for when filling was prevented at the onset of relaxation by making relaxation isometric, the pressure drop below the zero level of the system was magnified. Other considerations are discussed which also disprove that it is an artefact.

4. The minimum elastic state of the ventricle can be expressed by a smooth curve, along which the $\frac{\text{pressure}}{\text{volume}}$ ratios of the relaxed heart fall, regardless of whether the diastolic volume or the diastolic pressure is set.

5. The drop of pressure during relaxation below that set by the reservoir is taken as evidence that the ventricle is drawing in fluid.

6. These experiments are not contradicted by those on skeletal muscle suspended on mercury.

7. The sources of energy for the sucking action are mainly potential stores, created by the contraction process, such as energy of position and of elastic state. The distinction is made between the source of energy for the sucking action and for the flow of fluid. The distinction is shown to be the same as in an aspirating pump.

8. The conclusion is drawn that relaxation is of similar nature to contraction. Both are dependent on some reversible physico-chemical process which alters the elastic state of the muscle between two limits, the minimum for the relaxed and the maximum for the contracted heart.

9. Evidence is given to show that the ventricle not only can but does exert a sucking action in the intact mammal.

[2] An idea of the possible relative importance of the passive filling compared with that due to sucking action may be indicated by comparing the gradient of the volume curve during diastasis with that occurring during rapid inflow. If we assume that the gradient of filling due to the accumulation of the blood returning to the veins is of a similar order during rapid inflow as it is during diastasis, which is probably correct, then the sucking action of the ventricle is of considerable importance in filling since it changes the gradient of filling tremendously. This argument is suggestive but, frankly, not conclusive.

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OBSERVATION UPON THE BLOOD FLOW THROUGH SKELETAL MUSCLE BY THE USE OF THE HOT WIRE ANEMOMETER[1]

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Many observers have determined the venous outflow from muscles during contraction. Notably among these are Sadler (1869), Gaskell (1878), Burton-Opitz (1903), Verzar (1912) and Barcroft and Kato (1915). They determined the alterations in the outflow, caused by the contraction, by counting the drops or by measuring the amount in calibrated tubes or by the use of a stromuhr. Studies were not performed upon the arterial inflow.

In the present work, the effects of contraction upon the arterial inflow and venous outflow were determined. The instruments which were used were extremely sensitive and small alterations in flow were detected. Changes in arterial inflow will be chiefly emphasized in this paper.

Method. In some experiments the posterior extremities of the frog were used and in others the gastrocnemius of the dog. In the studies upon frogs, a cannula was placed in the terminal abdominal aorta and the legs were perfused with Ringer's solution from a Woulfe's bottle. A string connected the toes of the animal to a lever which was placed in front of the camera in order that the contractions could be photographed. The effects of both single and tetanic stimulation were studied.

In the experiments upon dogs, the gastrocnemius was prepared so that the muscle could be perfused through one cannulated artery and the outflow could be collected from one vein. The other arteries and veins were ligated. The gastrocnemius was left in its normal position except for freeing the tendo-achilles so that it could be attached to a lever in order to register the contractions during stimulation. The perfusion fluid consisted of defibrinated blood which was oxygenated. This was placed in a Woulfe's bottle. A warming coil placed near the inflow cannula maintained the temperature of the blood at approximately that of the body. The general arrangement of the perfusion apparatus is shown in figure 1.

[1] This work was performed in the Physiological Laboratory, Cambridge University, Cambridge, England under the direction of Dr. G. V. Anrep.

The dogs remained alive during the experiments. Ether or chloralose was used as the anesthetic.

The principle of the hot wire as described by Tucker (1921) was used in these experiments. A spiral platinum wire about 12 mu in diameter was mounted in a metal cylinder 1 mm. in diameter. The platinum wire had

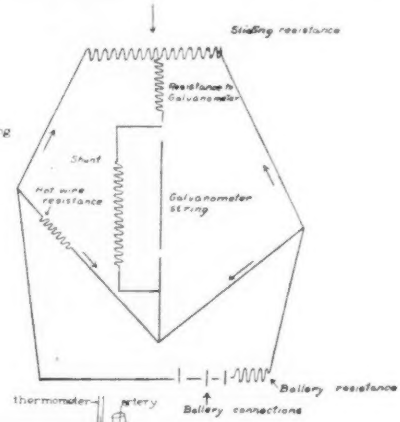

Fig. 2

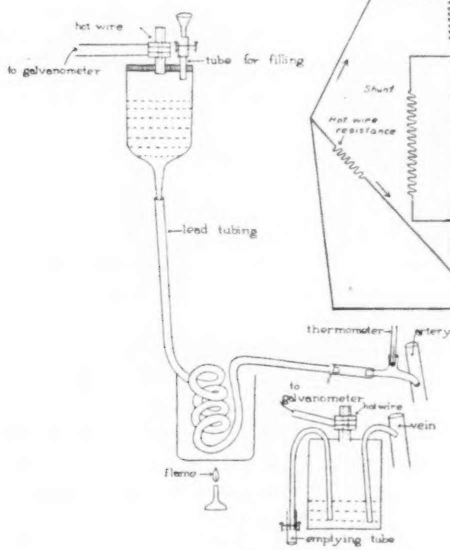

Fig. 1

Fig. 1. General arrangement of apparatus. Inflow bottle above. When the tube which is used in filling the bottle is closed, air can enter only through the hot wire container. Outflow bottle below. When the emptying tube is occluded, the air which is displaced by the entering fluid can escape only through the hot wire container.

Fig. 2. General arrangement of Wheatstone bridge. The hot wire occupies one arm. It is so adjusted that with the hot wire heated and when the base line is taken that the two arms are balanced, no current flows through the string. The cooling of the wire by air results in a disturbance of this balance and a deflection of the galvanometer string.

a resistance of about 30 ohms when raised to a dull red heat by means of a battery of approximately 10 volts. The hot wire forms one arm of a Wheatstone bridge. The general arrangement of this is shown in figure 2. Due to the low heat capacity of the wire, it is cooled very rapidly even by small currents of air. A string galvanometer with copper strings 10 to 12

mu in diameter was used for recording the changes in flow. The sensitivity of the string which has a complete period of 0.003 second was such as to give a deflection of 5 to 20 mm. for 3 millivolts. The magnification which was used was varied, it being enormously high when experimenting with the frog's legs.

A steady outflow of fluid from the Woulfe's bottle initiates a constant downward displacement of air around the hot wire and therefore a constant cooling and constant change in its electrical resistance. The hot wire can therefore be calibrated for known rates of flow or for known volumes of fluid introduced. If the outflow is greater than 1 cc. per second, a slight correction is necessary. The limit can be extended somewhat by using in the galvanometer a slacker fibre with a greater internal resistance. The flow in our experiments was much smaller, being about 3 cc. per minute when frogs were used and about 10 cc. per minute in the experiments upon dogs. The accuracy of the apparatus was determined by allowing known volumes of fluid to flow from the bottle and measuring the area of the record. The actual error was thought to be less than 3 per cent.

The same principle is involved when fluid is allowed to flow into the Woulfe's bottle. The air is displaced by the fluid and it results in a cooling of the hot wire and hence a change in its electrical resistance.

There is a slight lag in the heating and cooling of the hot wire which makes it incapable of responding entirely accurately to rapid changes in air velocity. The method of correction is similar to that used for a capillary electrometer. The deflection caused by either a sudden heating or cooling of the wire has the same lag. When it is desired to determine the absolute flow for a given period of time, correction for the lag is not necessary for the areas for the corrected and uncorrected curves are the same. It is obvious that the hot wire registrations cannot be used when the flow varies in both directions since in whatever direction the air current proceeds, the cooling of the hot wire will always be registered by a deflection of the galvanometer string in the same direction.

Simultaneous studies upon the inflow and outflow were not performed. In most of the experiments, observations upon the inflow were alternated with those upon the outflow.

In most of the experiments, the nerve or nerves to be stimulated were cut across. Studies before and after this procedure showed that the arterial inflow and venous outflow were greatly increased by division of the nerves to the muscles.

Results. *Arterial inflow.* The experiments upon the posterior extremities of frogs and upon the gastrocnemius muscle of dogs gave similar results. Single stimuli of sufficient strength to cause a vigorous contraction of the muscles resulted in a marked diminution of the arterial inflow. The onset of the diminution in the flow begins simultaneously with the

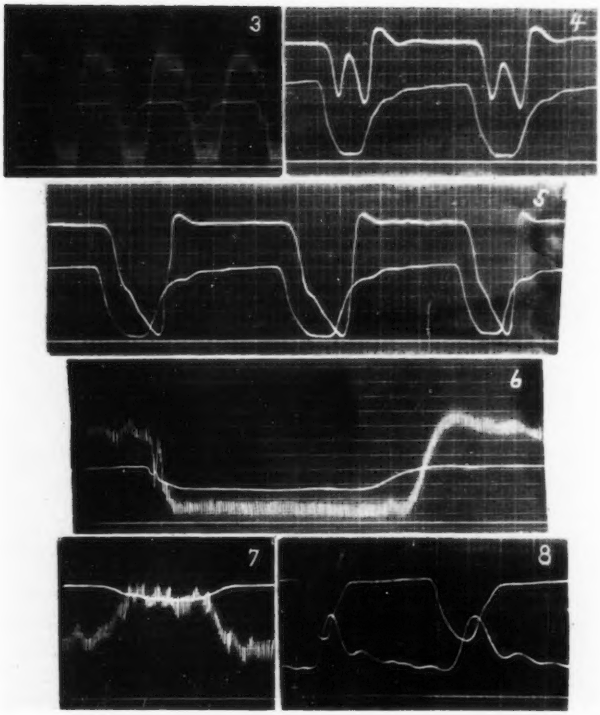

Fig. 3. Showing the effects of rhythmic stimuli upon the arterial inflow into the posterior extremities of a frog. Almost complete cessation of flow during the contraction. Base line at bottom. Record of contraction of muscles in centre. Smallest divisions represent 0.04 second in time. This and all the remaining records are to be read from left to right.

Fig. 4. The effects of rhythmic stimulation upon the arterial inflow into the gastrocnemius of a dog. Base line at bottom Muscle lever in centre. Weight attached to tendo-achilles was 280 grams. Perfusion pressure 103 cm. H_2O. This and all the remaining records with the exception of figure 7 are from experiments on dogs. Record retouched.

Fig. 5. Showing the effects of rhythmical stimuli upon the arterial inflow when a valve prevented the back-flow of fluid into the Woulfe's bottle. Almost complete cessation of flow during each contraction. Base line at bottom. Muscle lever in centre. Galvanometer string at top.

Fig. 6. Showing the effect of tetanic stimulation of short duration upon the arterial inflow. Marked decrease in flow during stimulation and increase after release. Weight 10 grams. Base line at bottom. Muscle lever in centre. Record retouched.

Fig. 7. The arterial inflow had been decreased markedly by tying weights amounting to 125 grams to the frog's feet. The weights were then lifted and the increased flow is shown. Base line at bottom. Galvanometer string in centre and muscle lever at top. Record retouched.

Fig. 8. Showing the effects of rhythmic stimuli upon the venous outflow. The outflow increases during the contraction and decreases after the muscle relaxes, the opposite of the effect upon the arterial inflow. Base line at bottom, galvanometer string in centre and muscle lever at top.

contraction of the muscles. The effects of rhythmical contractions of the posterior extremities of a frog are shown in figure 3. The strength of the stimuli was varied slightly. The arterial inflow almost completely stops during each contraction, returning to approximately its previous level between the contractions. The effects of two contractions of the gastrocnemius of the dog are shown in figure 4. The flow was slightly greater after each contraction than it was during the period preceding the stimulation. It is to be noted in figures 3 and 4 that the galvanometer string approaches the base-line during the early part of the contraction, that there is then a "back-shoot" towards the original level, which is followed by another and more marked approach to the base-line. The cause for this was not apparent at first. A valve was placed in the tubing leading from the Woulfe's bottle to the artery and the "back-shoot" was eliminated. A record obtained with the valve in is shown in figure 5. Without the valve, the vigorous contraction of the muscle forced fluid back into the Woulfe's bottle. This fluid which reëntered the bottle displaced air through the hot wire and cooled it.

The effect of short tetanic stimuli is demonstrated in figure 6. The flow almost completely ceased during the stimulation and returned to a level greater than that before the contraction after the stimulation was stopped. If the stimulation is continued until the muscle becomes fatigued, the inflow increases to an amount greater than that which existed before the contraction. The length of time during which this increase persists was not determined.

It seemed likely that the mechanical contraction of the muscle was probably largely responsible for the diminution in the arterial inflow which was found. In several experiments, sufficient weights were attached to the string which was connected to the muscle to almost completely stop the arterial inflow. At intervals the pull of the weights was released. The effect is shown in figure 7. It is to be seen that the flow increased immediately when the weights were removed and decreased immediately when the pull was again made on the muscle. No stimulation was applied in these experiments.

However, the alteration in the arterial inflow following stimulation was not due entirely to the mechanical shortening of the muscle. This was demonstrated in incompleted experiments in which the muscle was completely paralyzed by curare. Stimulation of the nerve to the muscle produced a small alteration in the inflow even though there was no contraction of the muscle.

Venous outflow. The immediate alterations in the venous outflow which were caused by stimulation of the motor nerve to a muscle were just the reverse of the changes in the arterial inflow. Stimulation of the nerve was

followed by an immediate increase in the outflow. Relaxation of the muscle after a single stimulus was associated with a decrease in the venous outflow. When strong tetanic stimuli were applied, there was first an increase in outflow which was followed by almost complete cessation of flow. After the muscle became fatigued, the venous outflow increased as did the arterial inflow. The effects of single stimuli upon the venous outflow are shown in figure 8.

A comparison of the present findings with those obtained by Anrep, Cruickshank, Downing and Rau (1927) in their studies on the coronary circulation shows that contraction of cardiac and skeletal muscles produces similar alterations upon the arterial inflow and venous outflow of blood. They state, "The outflow from the drained coronary sinus is increased during systole and consists of three waves; the first closely following the auricular contraction, the onset of the second occurring simultaneously with the beginning of the ventricular contraction, and the third during the ventricular contraction. The inflow into the coronary arteries is diminished during systole."

SUMMARY

1. The alterations in the flow of blood to and from skeletal muscles which were caused by stimulation of the motor nerve have been determined by the use of the hot wire anemometer.

2. Muscular contraction produced by a single stimulus produces a momentary diminution in arterial inflow and increase in venous outflow.

3. Contraction as a result of tetanic stimuli is associated with a decrease in arterial inflow as long as the muscle remains contracted and an increase in outflow at the beginning which is followed by an almost complete cessation of flow. Relaxation of the muscle is followed by a greater flow than existed before the stimulation.

4. If the muscle is stimulated until it becomes fatigued, there is an increase in both arterial inflow and venous outflow.

5. Division of the nerve to a muscle greatly increases the flow of blood through it.

6. If sufficiently heavy weights are suspended from the tendon of a muscle, the flow of blood through the muscle almost completely stops. The flow increases when the weights are lifted.

7. Although the vasomotor nerves are responsible for a small part of the alterations in flow produced by stimulation of the nerves to muscles, the major effects are due to the mechanical shortening and lengthening of the muscle fibers.

8. The effects of contraction upon the flow of blood are similar in cardiac and skeletal muscles.

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THE EFFECT OF A SOLUTION OF ACACIA IN RESTORING DIMINISHED BODY FLUID[1]

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Solution of acacia was used in cases of Asiatic cholera by Rogers (1919) with disappointing results. It occurred to us that the reason for these disappointing results might be that the solution of acacia restores volume of blood but fails to furnish adequate fluid to the extravascular tissues. A ready method of studying this problem was offered by the simple procedure for producing experimental dehydration, previously described by Keith (1924).

Methods. Healthy adult female dogs, immunized to distemper, were used. An attempt was made to select animals that were not pregnant. The approximate weight was 10 kgm. Some of the dogs were used more than once, but at least three weeks elapsed between experiments. Dogs without albuminuria were used.

Twenty hours before beginning an experiment, food and water were removed from the cage. The dog was given 300 cc. of water by stomach tube, so that each experiment was begun under the same conditions.

Food or water was not given on the day of the experiment, excepting the solutions injected in the saphenous vein. All intravenous injections were given at a continuous, measured rate, with a Woodyatt automatic pump, using sterile apparatus and specially prepared rubber tubing (Stokes and Buseman, 1920). Rubber tubing attached to a metal retention catheter was arranged to drain into a graduated cylinder, so that the secretion of urine could be constantly measured. The urine passed during the following night was measured by keeping the animal in a metabolism cage. The animal was weighed at various stages of the experiment. Specimens of blood were obtained by jugular venepuncture for determinations of the hematocrit value, concentration of hemoglobin, and concentration of urea. The initial blood volume was calculated as 100 cc. for each kilogram of body

[1] Abridgment of thesis submitted by Maurice A. Walker to the Faculty of the Graduate School of the University of Minnesota in partial fulfillment of the requirement for the degree of Master of Science in Surgery, 1930.

weight, although it has been shown that the blood volume is more variable in the dog than in man (Brown and Rowntree, 1929).

The solution of acacia used in all these experiments was prepared according to Osterberg's technic as described by Huffman (1929). This was a solution of 6 per cent acacia and 0.9 per cent sodium chloride.[2] Two per cent and 4 per cent solutions of acacia were prepared by dilution of the 6 per cent solution with 0.9 per cent solution of sodium chloride.

Results. *Simple injection.* Before using solution of acacia to restore the fluid lost after dehydration, we thought it advisable to study the effect of large quantities of solutions of acacia and of sodium chloride injected into normal animals. After the injection of as much as 4.8 grams of acacia for each kilogram of body weight of the dog, there was no evidence of toxic effect, either at the time of the experiment or in the following days. The data are given in table 1.

During the period of twenty-four hours after beginning injection of solution of sodium chloride the volumes of urine secreted in the two experiments were 6.5 and 9.2 per cent, respectively, of the initial body weight of the dog, or 81 and 115 per cent of the volume of solution injected. After injection of solution of acacia, the secretion of urine varied from 5.1 to 8.2 per cent of the initial body weight, or 64 to 102 per cent of the volume injected (table 1). These results are opposite to those reported by Knowlton (1911) and Kruse (1919 and 1920).

Dehydration. Fifty per cent solution of sucrose was injected at the rate of 7 grams of sucrose an hour for each kilogram of body weight, for two hours. A period of four hours was then allowed for diuresis to subside. The loss of fluid was considered to be the total amount of urine secreted since the beginning of the experiment, minus the amount of solution of sucrose injected. After dehydration, the average value for hemoglobin was 127.4 per cent, a calculated decrease in blood volume of 21.5 per cent. In experiments previously reported by Keith (1924) and Keith and Whelan (1926), after dehydration by the injection of 50 per cent solutions of sucrose and of dextrose, the average loss of fluid was 8 per cent of the initial body weight and the average value for hemoglobin was 125 per cent. Further data are given in table 2.

Often the dog had a formed bowel movement in the course of the injection of sucrose. The buccal mucous membranes were dry at the end of the period of loss of water, and the dog usually was drowsy and listless.

In ten experiments, the loss of fluid was restored with two solutions injected in immediate sequence. The calculated average decrease in blood volume (20 per cent of the initial blood volume) was replaced with solution

[2] This solution contains approximately 56 mgm. of calcium and 143 mgm. of reducing substances in each 100 cc. and has a pH value of 4.6.

TABLE 1

Results of simple injection of solution of acacia and of sodium chloride

DOG	BODY WEIGHT	SOLUTION INJECTED				URINE TWENTY-FOUR HOURS AFTER BEGINNING OF INJECTION		ALTERATIONS IN BLOOD								
								Hemoglobin, per cent			Hematocrit, per cent of cells			Urea, mgm. in each 100 cc.		
			Per cent	Amount	Per cent of initial body weight	Cc.	Per cent of initial body weight	Initial	Immediately after injection of solution	Twenty-four hours after the beginning of injection	Initial	Immediately after injection of solution	Twenty-four hours after the beginning of injection	Initial	Immediately after injection of solution	Twenty-four hours after the beginning of injection
	kgm.			*cc.*												
1	10.34	Sodium chloride	0.8	830	8.0	670	6.5	100	79	93	31	26	32	47	39	36
2	9.09	Sodium chloride	0.8	727	8.0	835	9.2	100	93	100	40	41	42	17	19	9
3	8.85	Acacia	2.0	708	8.0	455	5.1	100	67	86				40	37	32
4	18.74	Acacia	4.0	1,500	8.0	1,530	8.2	100	59	91				10	13	14
5	7.14	Acacia	4.0	575	8.0	469	6.6	100	70	79	31	22	27	17	13	23
1	9.08	Acacia	6.0	730	8.0	715	7.9	100	53	70	38	20	27	24	23	24

TABLE 2

Results of dehydration and of restoration of fluid

DOG	BODY WEIGHT	SOLUTION INJECTED FOR RESTORATION OF FLUID LOST							HEMOGLOBIN, PER CENT						
		First solution			Second solution			Total volume injected	Initial	After dehydration	After first injection	After total injection	After beginning of injection of solution of sugar		
			Per cent	Cc.		Per cent	Cc.						24 hours	48 hours	72 hours
	kgm.							cc.							
6	8.87	Sodium chloride	0.8	555	Acacia	6.0	180	735	100	123	83	76	97	100	
6*	8.55	Sodium chloride	0.8	305	Acacia	6.0	171	476	100	127	96	79	98		
7	10.99	Sodium chloride	0.8	1,030	Acacia	4.0	220	1,250	100	134	84	75	110		
8	9.47	Acacia	6.0	200	Sodium chloride	0.8	650	850	100	119	93	73	73	81	92
5	7.97	Acacia	6.0	160	Sodium chloride	0.8	500	660	100	128	106	70	83	100	
9	10.66	Acacia	5.0	225	Sodium chloride	0.8	550	775	100	131	105	75	89	80	88
10	10.59	Acacia	4.0	212	Sodium chloride	0.8	652	864	100	118	89	71	87	96	92
10*	11.06	Acacia	4.0	222	Sodium chloride	0.8	584	806	100	127	102	81	100	97	
3	9.72	Acacia	2.0	195	Sodium chloride	0.8	725	920	100	128	101	69	76	83	96
8	9.56	Acacia	2.0	200	Sodium chloride	0.8	700	900	100	124	100	70	73	86	100
6	9.78	Sodium chloride	0.8					675	100	116		83	96	95	95
1	11.47	Acacia	2.0					1,120	100	139		63	70	82	87
9†	9.75	Acacia	6.0					773	100	142		59			
11‡	11.15	Acacia	6.0					1,125	100	128		47	62		

* These dogs were dehydrated by injection of 50 per cent solution of dextrose instead of sucrose.

† Dog was found dead in cage the morning after experiment, no pathologic changes.

‡ Dog died five days after experiment, no pathologic changes.

of acacia; the total amount was injected in thirty minutes. Solution of 0.8 per cent sodium chloride was injected at the rate of 10 cc. each minute to replace the remainder of the fluid lost, which theoretically came from the tissues of the animal.

From clinical observation of the animals, it made no difference whether the solution of acacia or of sodium chloride was injected first. By the time the injections were completed, the dogs were again alert, and the mucous membranes of the mouth were moist. None of the animals seemed harmed, either during the experiments or on succeeding days. When solution of sodium chloride was injected before acacia, the concentration of hemoglobin in the blood returned to practically normal within twenty-four hours. On the other hand, when the restoration of fluid lost was begun with solution of acacia the concentration of hemoglobin usually did not return to normal until the second or third day after the experiment. This delay did not appear to depend on the concentration of the solution of acacia.

TABLE 3

Experiments in dehydration in which the concentration of urea in the blood increased after injection of acacia

DOG	ACACIA INJECTED, GRAM FOR EACH KILOGRAM OF BODY WEIGHT	UREA, MGM. IN EACH 100 CC. OF BLOOD				
		Initial	After dehydration	After first injection	After second injection	Twenty-four hours after beginning of injection of solution of sugar
7	0.8	13	13	28	24	66
9	1.0	21	37	40		70
8	1.2	28	40	42		56

In only three instances was the concentration of urea in the blood increased significantly (table 3). Since this increase occurred whether solution of sodium chloride or of acacia was given first, and since similar and larger doses of acacia did not cause increase in concentration of urea, it is probable that other factors were responsible, such as the original method of dehydration as suggested in previous experiments by Keith (1924) and Keith and Whelan (1926). In the other experiments of this group, the concentration of urea never was more than 45 mgm. for each 100 cc. of blood.

In four dogs, the total amount of fluid lost by dehydration was restored by injection of a single solution (table 2). The animal which received 0.8 per cent solution of sodium chloride seemed normal after restoration of the fluid lost; the concentration of hemoglobin in the blood had returned to normal twenty-four hours after the experiment had begun, as previously has been noted by Keith (1924). The only apparent difference after

restoration with 2 per cent solution of acacia (1.9 grams of acacia for each kilogram of body weight) was the slower return to normal of the concentration of hemoglobin, that is, in six days. Restoration of the total loss of fluid with 6 per cent solution of acacia resulted in the deaths of two animals.

In the fourteen experiments with dehydration the volume of urine secreted during the sixteen hours following restoration of the loss of fluid varied from 95 to 350 cc., or from 1.0 to 3.2 per cent of the initial body weight of the animals, much less than the total volume of fluid injected. The volume of urine secreted did not seem to be affected by the amount of acacia injected.

Five dogs were dehydrated by injection of sugar dissolved in solution of acacia. The loss of fluid was restored by injection of 0.8 per cent sodium chloride solution. The results are given in table 4.

Both dogs that died had convulsions. They were similar to those previously described by Balcar, Sansum and Woodyatt (1919) and by Keith (1924) as occurring during the injection of concentrated solutions of sugar to produce diuresis, and were accompanied by rapid elevation of body temperature, persistent rigor, and death. The amount of acacia injected in the dogs that died was not nearly so much as was injected into dehydrated animals, without noticeable ill effects. Therefore, these deaths could probably be ascribed to the concentrated solution of sugar. It seemed definite that when the sugars were dissolved in 6 per cent solution of acacia, the amount which a dog could tolerate was less than when the sugars were dissolved in 4 per cent solution of acacia.

The presence of acacia in the solutions injected did not decrease the amount of fluid lost by diuresis. The loss of fluid varied from 5.2 to 10.4 per cent of the initial body weight; the average was 7.7 per cent. These results are similar to those reported by Mattill, Mayer, and Sauer (1920) in rabbits, when the dextrose solution contained 3 per cent acacia. The concentration of urea in the blood was not increased in our experiments, except in dog 12 (table 4).

Hemorrhage. In the experiments thus far described it was evident that injection of a solution of acacia into a dehydrated animal resulted in disturbance of the water equilibrium between the blood and the extravascular tissues. Therefore, in order to reduce the blood volume by an alternate method an acute hemorrhage was instituted in five experiments. The blood was removed in four or five nearly equal bleedings, fifteen or twenty minutes apart so that the total hemorrhage occurred in approximately two hours, the same period as that required for an injection of solution of sucrose for dehydration. The results are given in table 5. In the experiment in which only 27 per cent of the estimated amount of blood in the body was removed, the animal (dog 15) apparently was not greatly

TABLE 4

Dehydration by injection of sugar dissolved in solution of acacia

Dog	Body weight	Solution injected for dehydration	Acacia, gram for each kgm. of body weight	Sugar injected gram for each kgm. of body weight, per hour for 2 hours	Loss of fluid		Alterations in blood										Remarks
							Hemoglobin, per cent					Hematocrit, per cent of cells					
					Cc.	Per cent of initial body weight	Initial	Immediately after injection of solution for dehydration	After loss of fluid	Immediately after injection of 0.8 per cent solution of sodium chloride	Twenty-four hours after beginning of injection of acacia-sugar solution	Initial	Immediately after injection of solution for dehydration	After loss of fluid	Immediately after injection of 0.8 per cent solution of sodium chloride	Twenty-four hours after beginning of injection of acacia-sugar solution	
	kgm.																
7	11.05	Sucrose 200 gm. in 4 per cent acacia solution to make 400 cc.	0.84	7	1,025	9.3	100	95	121	73	90	48	38	54	35	46	Normal
10	11.38	Dextrose 200 gm. in 4 per cent acacia solution to make 400 cc.	0.89	8	676	5.9	100	98	93	67	97	46	43	42	33		
7	11.55	Sucrose 200 gm. in 6 per cent acacia solution to make 400 cc.	0.91	5	884	7.7	100	100	113	82	95	43	43	51	37	44	
12	8.25	Sucrose 200 gm. in 6 per cent acacia solution to make 400 cc.	1.5	8	861	10.4	100	59	107	61	90	43	39	41	26	40	Died
13	10.46	Dextrose 200 gm. in 6 per cent acacia solution to make 400 cc.	1.34	8	540	5.2	100	86				46	36				Died

TABLE 5

Results of induced hemorrhage and restoration of fluid

DOG	BODY WEIGHT	SOLUTION INJECTED, PER CENT	AMOUNT OF BLOOD LOST		VOLUME OF FLUID INJECTED		VOLUME OF URINE		ALTERATIONS IN BLOOD							
									Hemoglobin, per cent				Hematocrit, per cent of cells			
			Cc.	Per cent of initial body weight	Cc. (at rate of 10 cc. each minute)	Per cent of initial body weight	Cc.	Per cent of initial body weight	Initial	Immediately after hemorrhage	Immediately after injection of solution	Twenty-two hours after beginning of injection of solution	Initial	Immediately after hemorrhage	Immediately after injection of solution	Twenty-two hours after beginning of injection of solution
	kgm.															
14	6.82	Sodium chloride, 0.8	320	4.7	320	4.7	183	2.7	100	84	68	55	50	31	32	29
10	11.23	Dextrose, 5	543	4.8	545	4.8	297	2.6	100	81	58	54	46		27	27
1	10.03	Acacia, 4	532	5.3	600	6.0	772	7.7	100	83	42	53				
15	13.65	Acacia, 6	370	2.7	370	2.7	425	3.1	100	113	77	80	45	48	35	35
16	7.97	Acacia, 6	450	5.6	450	5.6	620	7.8	100	76	35	56				

affected by the hemorrhage. The other dogs, however, were limp, cyanotic, and drowsy after the final bleeding.

An amount of fluid equal to the total amount of blood withdrawn was injected immediately after the last bleeding, at the rate of 10 cc. each minute. In one dog the blood was replaced with 0.8 per cent solution of sodium chloride. In another dog, 5 per cent solution of dextrose was injected. These two dogs continued in a state of shock and apathy resulting from the hemorrhage. They staggered when placed on their feet, and were content to lie half asleep. These dogs had no appetite for food or water on the days after the injections. The dog which received 0.8 per cent solution of sodium chloride died seven days after the injection; the dog which received 5 per cent solution of dextrose died on the fifth day. After injection of 4 per cent solution of acacia in one experiment, and 6 per cent solution of acacia in two experiments, the dogs seemed practically normal. They could walk, run, and bark, and were no longer cyanotic. They seemed thirsty, and when water was given the morning after the experiment, they drank with great avidity. These animals were observed for six months after the experiments, and at no time were any toxic symptoms observed, an experience which corresponds closely to that of Rous and Wilson (1918) in similar experiments on rabbits.

The concentration of urea in the blood was estimated in order to determine whether there was interference with its renal excretion. In dog 16, the concentration of blood urea increased slightly after hemorrhage. In dog 1 there was no rise after hemorrhage, but after injection of acacia the concentration of urea increased to 40 mgm. for each 100 cc. of blood. In dog 15, in which the amount of blood lost was only 27 per cent of the volume of blood in the body, there was no change in concentration of urea.

It should be noted that the volume of urine secreted in the period of twenty-four hours after the injection of isotonic solutions of dextrose or sodium chloride was less than the amount of solution injected. On the other hand, the diuresis after injection of solution of acacia was greater in every instance than the amount of solution injected (table 5). These diuretic results are only relative. Several investigators have shown that even in the totally fasting animal, a certain volume of urine is excreted daily.

Comment. If a large volume of solution of acacia was directly injected into normal dogs, the concentration of hemoglobin reached a lower value, and returned to normal more slowly, than after injection of an isotonic solution of sodium chloride. The effect of the two solutions on secretion of urine was practically the same. It is obvious that 6 per cent solution of acacia can be injected into a normal dog in the amount and manner employed in these experiments (4.8 grams of acacia for each kilogram of body weight) without toxic effects. The equivalent amount for a normal

man weighing 70 kgm. would be 5.6 liters of a 6 per cent solution of acacia. Similar experimental results were obtained in cats by Czerny (1894) and later in dogs by Erlanger and Gasser (1919), and Meek, Erlanger and Gasser (1919). It is apparent that acacia is more toxic in a dehydrated dog than in a normal dog. We found that injection of 4.8 grams of acacia for each kilogram of body weight, which did not cause untoward effect in the normal animal, resulted in death of a dehydrated dog. The largest amount of acacia injected after dehydration, with no toxic manifestation whatsoever, was 1.9 grams for each kilogram of body weight. Evidence of toxic effect was shown by a rising concentration of blood urea in three experiments after dehydration and replacement of the lost fluid by solution of acacia. The amounts of acacia injected were 0.8, 1.0, and 1.2 grams for each kilogram of body weight.

The dye method was not used for determination of blood volume in these experiments, but we feel that certain conclusions as to changes in blood volume can be drawn from the changes in concentration of hemoglobin and in hematocrit values (cell-plasma ratio). After simple injection of isotonic salt solution, even in large quantities, the concentration of hemoglobin returned to practically normal within a few hours. This has been observed both clinically and experimentally many times; a study by Chanutin, Smith, and Mendel (1924) exactly corroborated our results. After injection of solution of acacia, however, the concentration of hemoglobin was decreased, and in no case returned to normal before the third day. A similar result was noted by Czerny (1894) who used the number of erythrocytes in each unit of blood as an index to the changes in blood volume after injection of solution of acacia.

After dehydration, injection of isotonic solution of sodium chloride apparently resulted in rapid restoration to a normal distribution of the water in the blood and tissues. The result was the same when the lost fluid was partially restored by solution of sodium chloride, followed by solution of acacia. When solution of acacia was injected first, in an amount calculated to replace the decreased volume of blood, and was followed by injection of solution of sodium chloride to replace the remainder of the fluid lost, there was evidence suggesting retention of water in the blood. The relative changes in blood volume can be roughly calculated from the concentration of hemoglobin by use of the formula:

$$\text{Volume}_2 = \frac{\text{Hemoglobin concentration}_1}{\text{Hemoglobin concentration}_2} \times \text{Volume}_1$$

When the entire loss of fluid was restored with 6 per cent solution of acacia in the two animals that died, the increase in blood volume, as calculated in this manner, was greater in both cases than the actual amount of solution of acacia injected (table 6).

This result seems to indicate that injection of 6 per cent solution of acacia into a dehydrated dog attracts further water from the tissues into the blood. Certainly very little water escapes from the blood into the already dehydrated tissues. The results of these dehydration experiments emphasize the practical importance of restoring fluid to the tissues generally in the form of isotonic sodium chloride or dextrose solutions before injecting acacia solutions to restore the blood volume.

After hemorrhage, of course, relative changes in blood volume could be only roughly estimated. With the exception of dog 15, the volume of blood removed in each case was about half the estimated total blood volume of the animal. After injection of solution of acacia, the circulating volume of blood was definitely greater than after either isotonic solution of sodium chloride or of solution of dextrose. This conclusion was reached from comparison of the concentration of hemoglobin and the volume of the erythrocytes measured by the hematocrit. The arterial pulse was strikingly stronger in dogs after injection of solution of acacia than in dogs in which isotonic solution of sodium chloride or of solution of dextrose was injected. In the period of twenty-two hours following injection of solution of sodium chloride or of dextrose, the concentration of hemoglobin continued to decrease. Twenty-two hours after injection of solution of acacia the concentration of hemoglobin had slightly increased, probably because of the greater loss of water by secretion of urine. The concentration of hemoglobin remained at about 50 per cent of the initial value, but the animals seemed normal.

TABLE 6

Calculated changes in blood volume after injection of 6 per cent solution of acacia into dogs after dehydration

DOG	INITIAL BLOOD VOLUME	CALCULATED BLOOD VOLUME AFTER DEHYDRATION	VOLUME OF SOLUTION OF ACACIA, INJECTED	SUM OF TWO COLUMNS TO IMMEDIATE LEFT	BLOOD VOLUME AFTER INJECTION, CALCULATED FROM CONCENTRATION OF HEMOGLOBIN
	cc., estimated	*cc.*	*cc.*	*cc.*	*cc.*
9	975	687	773	1,460	1,653
11	1,115	871	1,125	1,996	2,372

SUMMARY

Intravenous injection of a volume of 6 per cent solution of acacia equal to 8 per cent of the body weight of a dog (4.8 grams of acacia for each kilogram of body weight) did not cause manifestations of toxicity and resulted in secretion of practically the same volume of urine as injection of a similar quantity of 0.8 per cent solution of sodium chloride, but the

concentration of hemoglobin in the blood remained decreased for at least three days.

After dehydration of dogs by intravenous injection of concentrated solutions of sugar, the largest amount of acacia injected without evidence of toxicity was 1.9 grams for each kilogram of body weight. Amounts of 4.8 grams for each kilogram of body weight caused death.

Dogs were dehydrated also by intravenous injection of sugars dissolved in solution of acacia. Except when the quantity of one or the other substance was great, the results were not noticeably different from those obtained when solution of sugar alone was used for dehydration.

After loss by hemorrhage of approximately 50 per cent of the total amount of blood in the body the fluid lost could not be restored with isotonic solution of sodium chloride or of solution of dextrose, and the animals died. However, solution of acacia restored the blood volume and the animals remained well, although the concentration of hemoglobin was greatly reduced. The volume of urine secreted in twenty-two hours was greater than the volume of solution of acacia injected. The general results of these experiments indicate that solution of acacia has the property of holding the injected fluid in the circulation for a considerable period. It does not inhibit renal excretion of water. There is evidence that under certain conditions acacia not only holds water in the blood, but at the same time favors renal excretion of water of extra-vascular origin.

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REVERSAL IN THE CROSSED EXTENSION REFLEX IN DECEREBRATE, DECAPITATE AND SPINAL CATS

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In an endeavor to analyze the various reflex components in the limb movements of experimental animals, it has been the practice to elicit reflex responses by stimulating the central end of a cut peripheral nerve with repetitive induction shocks. In this type of stimulation, the fibers of the nerve are activated without respect to the type of sensation they mediate, i.e., the various types of exteroceptive and proprioceptive afferent fibers are stimulated simultaneously provided the stimulus is adequate to bring the small as well as the large fibers into activity (Gasser and Erlanger, 1927). Although at best this is an unnatural stimulus, it is the only method we have of controlling the strength, duration, and frequency of stimulation in an accurate manner.

According to the general law of reflex contraction, it is stated that (Fulton, 1926, p. 295), "stimulation of the central cut end of an afferent nerve supplying the hind limb of one side causes: (1) reflex contraction of the flexors on the same side and inhibition of the extensors, and (2) contraction of the extensors of the opposite side and inhibition of the flexors." There are a number of exceptions to this law which are known but which are often neglected in a consideration of reflex responses.

The reflex response varies, with the neural balance (Graham Brown, 1911a), the character of the electrical stimulus (Sherrington and Sowton, 1911a, b; Graham Brown, 1911b, 1912; Graham Brown and Sherrington, 1912; Beritoff, 1923a, b), the passive posture of the limbs (Gergens, 1876; Sherrington, 1900 and 1905b; von Uexküll, 1904; Magnus, 1909a, b, 1910a, b; Graham Brown, 1911a), the posture of the head and neck (Socin and Storm van Leeuwen, 1914; Beritoff, 1915; Girndt, 1926; Pollock and Davis, 1927; and Pi-Suñer and Fulton, 1929), the administration of strychnine, chloroform, or ether (Sherrington, 1905a; Owen and Sherrington, 1911; Sherrington and Sowton, 1911c; Graham Brown, 1914; and Forbes, 1921), and the onset of fatigue (Sherrington and Sowton, 1911b; Verzar, 1923). It is with some of these factors governing reflex reversals in the crossed extension reflex that we are concerned in this paper.

METHOD. The reflexes at the ankle joint have been recorded in 19 spinal, 4 decapitate and 14 decerebrate cats. The gastrocnemius and tibialis anterior muscles were attached to muscle levers weighted with rubber bands and the movements of the tendons were magnified seven times in the tracings. The stimuli used were of three types; 1, rapid faradic induction shocks with both makes and breaks present (both Harvard and DuBois Reymond coils were used); 2, slow break shocks from a Harvard coil (makes were short-circuited and frequency was controlled through a motor-driven commutator), and 3, slow makes and breaks from a Harvard coil (frequency regulated by a Harvard interrupter). Between 1.9 and 2.0 amperes were delivered to the primary coil. The stimuli were applied to the nerve through shielded platinum electrodes. During the course of the experiments, different nerves were stimulated, the tibial at the ankle, the saphenous, and the femoral at its entrance to the limb before it has broken up into its various muscular branches.

The spinal cats were utilized from 1 to 15 days following transection. The decapitate and decerebrate animals were acute preparations. Both anemic (Pollock and Davis, 1924) and transection decerebrations were used. In the latter, the sections were made at the intercollicular level. The operative technique, the care of the spinal animals, and the methods used in making the observations and tracings have been described in a previous paper (Ranson and Hinsey, 1930).

Intervals of a least five minutes and in many cases ten minutes were allowed between successive responses so that "spinal induction" (Sherrington, 1905b) and fatigue could be eliminated as causative factors in the reversals. In selecting responses for comparison from the standpoint of reflex reversals, care has been exercised to take them from similar intervals in the experiment. The animals were all placed in the dorsal position with the heads fixed in position, and the fore-limbs and stimulated ipsilateral hind-limbs tied in extension. In this way we have endeavored to remove as far as possible the effect of labyrinthine and neck reflexes as well as other postural factors as causes for the reversals which were seen.

OBSERVATIONS. *Transection decerebrations.* In these four preparations, the crossed reflexes were typically extensor in type. With moderate and strong stimuli, post-excitatory extensor rebound and rebound in the flexors was seen at times early in the experiment and, late in the experiment, it was possible to obtain flexor cocontraction with strong stimulation in two of the four experiments. Secondary coil separations of 6, 8 and 10 cm. were necessary to do this. In all cases but one, the extensor response was by far the most marked in the cocontraction. In this one exception, very late in the experiment ($6\frac{1}{2}$ hours after transection) with the secondary coil separated 6 cm., the crossed response was a cocontraction with flexors showing greater contraction and there was a marked post-excitatory flexor rebound which was rhythmical at first.

In one experiment, there was one instance of rate reversal. With the Harvard coil at 6 cm. separation, stimulation of the tibial nerve at the ankle gave pure crossed extension with one make and one break shock while with automatic rapid interruption at the same coil position there was cocontraction (more marked contraction in the extensors) with rhythmic rebound in the flexors and extensors. No evidence of a different response elicited from the saphenous and tibial nerves was obtained.

Anemic decerebrations. The animals decerebrated by the anemic method showed predominantly extensor responses but in four of nine experiments flexor type responses were seen at some time during the experiment. The latter were seen in several instances early in the experiments but as a rule they were better developed later on. It should be stated that, at the times when these reversals were seen, there was no marked diminution of extensor tonus and there was well developed decerebrate rigidity present. The reversals of response which were seen in these preparations were elicited in three ways: 1, by variations in the strength of stimulation; 2, by variations in the frequency of stimulation, and 3, by changing the nerve stimulated. These reversals were not obtained in all the preparations but they occurred frequently enough to attach some significance to them.

1. *Strength of stimulation.* Weak stimuli elicited as a general rule crossed extension with no flexor participation (if any flexor response was present, it was relaxation). When the strength of stimulus was increased, the flexors showed alternate or cocontraction with the extensors and at times the reversal showed pure crossed flexion. Figure 1 shows crossed extension elicited by stimulation of the tibial nerve at the ankle with rapid faradic shocks (50 makes and breaks a second) with the secondary coil of a Harvard inductorium separated 13 cm. and tilted at an angle of 85°. The next response of crossed flexion (fig. 2) was obtained five minutes later with the same conditions of stimulation with the exception that the coil was brought up to the horizontal at 13 cm. The second stimulus was perceptible but not painful to the tip of the tongue. Another experiment illustrated the variations in response which can be obtained by varying the strength of stimulus to the tibial nerve at the ankle. These responses were taken in succession five minutes apart early in the experiment. The stimuli were all rapid faradic induction shocks from a Harvard coil and the only variation in experimental procedure was in the position of the secondary coil. With the secondary at 13 cm. and tilted at 85° there was pure crossed extension with flexor relaxation, at 13 cm. (horizontal) cocontraction with marked extension and slight flexion, at 10 cm. cocontraction was still present but the flexor contraction was greater than before, and at 6 cm. there was crossed flexion with alternate extension which started just before the stimulus stopped. This shows very well the absence of demarcation between flexion and extension and also that increasing the strength of stimu-

lation predisposes to more flexor participation. These reversals with variations in the strength of stimulus were seen in six of the nine experiments and were obtained from both the tibial nerve at the ankle and from the femoral nerve but not from the saphenous which, as we shall see later, predisposes to extension in these anemic decerebrate animals.

2. *Influence of the nerve stimulated.* It was possible to obtain reflex reversals in these preparations by keeping the stimulus constant and varying the nerve stimulated. The tibial at the ankle and the femoral showed flexor involvement provided the stimulus was strong enough while the crossed responses from the saphenous nerve were predominantly extensor. In figures 3, 4 and 5, the reflexes were all obtained with the same strength of stimulus which was a strong one, faradic shocks from a Harvard coil at 8 cm. secondary separation. In figure 3 there is crossed flexion with a very slight extensor cocontraction and extensor rebound on stimulation of the tibial nerve at the ankle, in figure 4 pure crossed extension from the saphenous nerve and in figure 5 crossed flexion accompanied by a very slow extensor contraction which was followed by a marked post-excitatory extensor rebound obtained from the femoral nerve. This stimulus was strong enough to excite all of the fibers in the respective nerves. The excitatory influence on the flexor half-center must be greater on stimulation of the tibial and femoral nerves than it is from the saphenous nerve under the conditions of neural balance that were present in these anemic decerebrate animals. The crossed responses from the saphenous nerve were always predominantly extension and only seldom there was slight flexor cocontraction or flexor rebound present. Reversals of this type were seen in seven of the nine experiments.

3. *Frequency reversals.* In four of seven experiments in which it was tried, it was found possible to obtain reversals by varying the frequency of stimulation, keeping the strength and other factors constant. Slow stimulation of the tibial nerve at the ankle and of the femoral nerve predisposed to extension while more rapid ones with the same strength showed flexor participation. Comparison of figure 3 (rapid faradic) with figures 6 (1 make and 1 break shock) and 7 (7 makes and 7 breaks per second) shows this reversal from flexion to extension in reflexes from the tibial nerve at the ankle. A similar reversal from the femoral is shown in figures 5 (rapid faradic) and 8 (slow makes and breaks). The makes and breaks were delivered from a Harvard coil with the secondary coil at 8 cm. and the only difference was that the automatic interrupter was used with rapid stimulation and a Harvard interrupter with the slower ones. We cannot say whether or not there was a difference in the configuration of the individual makes and breaks in the more rapid and the slower shocks.

Decapitate cats. The decapitate cats were under artificial respiration and were acute preparations, in this respect differing from the chronic low

spinal cats. Weak stimuli gave crossed extension which was reversed into crossed flexion with increasing strengths. This was seen in three of the five preparations. Reversals on changing the nerve stimulated were seen in four of five experiments. The tibial at the ankle tended to give crossed flexion (fig. 9) while stimulation of the saphenous elicited crossed extension (fig. 10). In only one experiment was an attempt made to study the effect of changing the frequency of stimulation. Here with rapid faradic as well as with slow break shocks, crossed flexion was seen. Thus we have no evidence of a frequency reversal in decapitate cats.

Spinal cats. By examining the type of responses seen in animals sectioned at different levels in the spinal cord, it has been shown that at levels from L 3 to T 4 there is no essential difference in the type of reflex responses seen in the kymographic tracings from animals transected at the different levels (Ranson and Hinsey, 1930). A great variety of responses was found to be present. In our original series we did not examine carefully reflex reversals in the kymographic tracings so we have made a subsequent examination in 19 spinal cats transected at varying levels from T 11 to T 4 from 1 to 15 days before the experiment. Following the examination of the responses in the undissected limb, the animal was decerebrated by the anemic method and then the muscles were exposed and prepared for kymographic tracings in a manner which we have described in the previous paper. The discussion will be confined to reversals in the crossed responses.

Reversals in intact limbs. In a series of 16 of the spinal cats, the animal was first suspended in a hammock. The tibial nerve at the ankle was exposed and faradic stimulation from a DuBois Reymond coil was applied through shielded platinum electrodes. The strength of stimulation was increased from weak to strong and the responses in the crossed limbs were examined. Four of these showed crossed extension with all strengths of stimulation when examined 7 to 15 days after transection. In the kymographic tracings obtained following dissection, two of these four exhibited crossed extension responses with all strengths, and the other two showed reversals from extension to flexion on going from weak to strong stimulation. In five of the spinal animals, crossed flexion reflexes were elicited in the intact limb with all strengths of stimulations and after dissection three of these five continued to give crossed flexor type responses and two showed strength reversals. Four of these five animals which showed flexion responses in the intact limbs were experimented one day following transection and the fifth after an interval of 7 days. Seven of the 16 spinal cats, when examined 4 to 11 days after transection showed strength reversals in the intact limbs and, of these, four gave crossed flexion type responses in the kymographic tracings, two strength reversals and one crossed extension reflexes. It is evident that in the spinal cat with undissected limbs, the neural balance may be tilted toward extension, toward flexion, or it may be

Figs. 1–13. Tracings recorded by tibialis anterior (above) and gastrocnemius (below). Isotonic contractions. Between 1.9 and 2.0 amperes were delivered to the primary coil. Time in seconds.

Fig 1. Crossed extension reflex. Anemic decerebration (12/18/29). Tibial nerve. Harvard coil at 13 cm. and set at 85° (rapid faradic).

Fig. 2. Crossed flexion reflex. Anemic decerebration (12/18/29). Tibial nerve. Harvard coil at 13 cm. (rapid faradic).

at such an equilibrium that strength reversals may be observed. Furthermore, the neural balance may be shifted following the dissection so that the responses obtained in the tracings may not always exactly resemble those seen before dissection. However, it should be remembered that the posture with the animal in the hammock is vastly different from that with it on its back and this undoubtedly may explain some of the changes even in the spinal animal in spite of the fact that the tonic activity of the labyrinth and the neck reflexes is removed from the spinal centers. It is of interest that four of the five animals, which showed only crossed flexor responses in the undissected limb, were experimented one day following the transection. The interval following transection may influence the state of the spinal centers and there may be a shift from a flexor toward an extensor balance as the interval is prolonged, although this series is not large enough to prove the point.

Reversals in the isolated muscles. The responses were variable in the kymographic tracings and showed all gradations between flexor and extensor responses. In a series of 19 spinal animals sectioned at levels be-

Fig. 3. Crossed flexion reflex with slight extensor cocontraction and extensor rebound. Anemic decerebration (12/18/29). Tibial nerve. Harvard coil at 8 cm. (rapid faradic).

Fig. 4. Crossed extension reflex. Anemic decerebration (12/18/29). Saphenous nerve. Harvard coil at 8 cm. (rapid faradic).

Fig. 5. Crossed flexion reflex with extensor cocontraction and post-excitatory extensor rebound. Anemic decerebration (12/18/29). Femoral nerve. Harvard coil at 8 cm. (rapid faradic).

Fig. 6. Crossed extension reflex. Anemic decerebration (12/18/29). Tibial nerve. Harvard coil at 8 cm. (1 make and 1 break).

Fig. 7. Crossed extension reflex. Anemic decerebration (12/18/29). Tibial nerve. Harvard coil at 8 cm. (7 makes and 7 breaks per second).

Fig. 8. Crossed extension reflex. Anemic decerebration (12/18/29). Femoral nerve. Harvard coil at 8 cm. (about 1 make and 1 break per second).

Fig. 9. Crossed extension reflex. Decapitate cat 124. Saphenous nerve. Harvard coil at 13 cm. (rapid faradic).

Fig. 10. Crossed flexion reflex. Decapitate cat 124. Tibial nerve. Harvard coil at 13 cm. (rapid faradic).

Fig. 11. Crossed extension reflex with DuBois Reymond coil at 30 cm. and 28 cm. (rapid faradic). Five minutes later, crossed flexion with extension inhibition followed by alternate extensor contraction with the DuBois Reymond coil at 20 cm. (rapid faradic). Spinal cat 66 with transection at T 5, nine days after operation. Tibial nerve.

Fig. 12. Crossed extension reflex—saphenous nerve. Five minutes later crossed flexion reflex with extension inhibition followed by alternate extensor contraction—tibial nerve. Spinal cat 66 with transection at T 5, nine days after operation. DuBois Reymond coil at 28 cm. (rapid faradic).

Fig. 13. Crossed flexion reflex with extensor inhibition during stimulation and extensor rebound at cessation of stimulation. Spinal cat 66 with transection at T 5, nine days after operation. Tibial nerve. Harvard coil at 6 cm. (3 breaks per second).

tween T 4 and T 11, nine showed crossed responses of both flexor and extensor types during the experiment, seven gave only flexor responses and three only extensor types.

Strength reversals. Six of the nine animals in which both extensor and flexor type reflexes were seen showed strength reversals. These were seen with both the tibial and the saphenous nerves. They were not always complete, i.e., the reversal was not always from pure crossed extension to pure crossed flexion but it was from a crossed response in which the extensors predominated to one where flexion predominated. One might say that weak stimuli predisposed to pure crossed extension while increases in the strength of stimulus brought in flexion which was present either as an alternate contraction, a cocontraction, or pure crossed flexion. In figure 11, rapid faradic stimulation of the tibial nerve with the DuBois Reymond coil at 30 cm. was just barely at threshold for the crossed response, then at 28 cm. there was pure crossed extension, and at 20 cm. there was crossed flexion with concomitant extensor inhibition which passed over into an alternate extensor contraction. Figure 13 shows a response from this same animal later in the experiment where a slow strong stimulation produced pure crossed flexion with extensor relaxation during stimulation and an extensor rebound after the cessation of stimulation.

Nerve reversals. Of the nine spinal cats which showed reversals, eight showed variations in the reflexes on changing the nerve stimulated. It is of interest that in four of these eight, stimulation of the saphenous produced predominantly flexor reflexes while the tibial at the ankle gave extensor responses. In the other four the opposite was true. This shows how variable the reactions may be, how dependent they are on the state of the spinal centers, and how difficult it would be to construct a rule of crossed response which would fit all cases. In figure 12, the first tracing shows pure crossed extension from rapid faradic stimulation of the saphenous. The second tracing was taken five minutes later and here the same stimulus applied to the tibial nerve at the ankle produced alternate flexion and extension. Here again there is the initial inhibition of the extensors which gives way to an alternate extensor contraction on relaxation of the flexor muscle. In figure 13 this inhibition is maintained throughout stimulation and gives way to an extensor rebound at the cessation of stimulation.

Frequency reversals. In only one of the nine experiments where reversals were present was a frequency reversal seen. This was in cat 83, where strong faradic stimulation of the saphenous nerve gave pure crossed flexion and slow stimulation gave a small poorly maintained crossed extension with the same strength of stimulation. Apparently the neural balance in spinal animals is not favorable to frequency reversals as it was in the anemic decerebrate preparations.

Discussion. These experiments show quite clearly how variable reflex

responses may be and that under certain conditions which can be controlled experimentally it is possible to change the type of reflex response. One of the most important factors is the state of the spinal centers or "neural balance." In the transection decerebrate animal, it was tilted over to the extensor side while in the anemic decerebrate preparations, in spite of the fact that the animals showed good extensor rigidity, it was possible to elicit flexor responses at certain stages of the experiments. In the decapitate and spinal animals, there was great variation in that the balance was either tipped to the extensor or the flexor side or at times it appeared to be at a neutral point and there was "dilemma of reaction." As a rule, however, the extensor responses were much more difficult to obtain in the decapitate and spinal animals than in the decerebrate preparations.

With the information we have at hand, it is difficult to interpret the reversals obtained by varying the strength and frequency of the stimulus and changing the afferent nerve stimulated. One might be tempted to say that with weak stimuli, the large fibers would be stimulated and these might predispose to extension and that, when the strength of the stimulus is increased, smaller fibers mediating a different type of sensibility, i.e., pain, might be activated, thus bringing in flexion. There is another possibility in that the changes in response may be due to the number of incoming impulses of a similar kind reaching the central nervous system from a number of different receptive fields. It is difficult to say that a stimulus which is perceptible but not painful to the tongue does not activate pain fibers. Not until the action potentials of the nerve fibers (Gasser and Erlanger, 1927) are studied simultaneously with the recording of the reflex responses will it be possible to answer this question. Then it will be possible to say how much of the potential of the nerve is present and from that to determine approximately the sizes of the fibers which are active. It would be interesting to know whether the C-spike (Erlanger and Gasser, 1930) needs to be present in the potential of the impulses which bring in crossed flexion in the decerebrate animal.

Frequency reversals were demonstrable in some of the anemic decerebrate animals but it was difficult to obtain similar evidence in the spinal and decapitate animals. It occurred with strengths of stimulus which were strong enough to activate all of the fibers in the nerve and therefore must depend upon some central effect. Whether or not it is caused by some Wedensky effect (Forbes, 1922) which required the type of neural balance present in some of the decerebrate preparations, cannot be determined. The work of Tiedemann (1910) is of interest in this regard in that he found in the frog under strychnine a reversal from excitation into inhibition when he increased the frequency of afferent stimulation.

Reversal caused by changing the nerve stimulated is subject to at least two possible explanations. The fiber content of the saphenous nerve

differs from the tibial nerve at the ankle and from the whole femoral nerve in that the saphenous is a purely sensory nerve and does not contain proprioceptive fibers from skeletal muscle while the femoral and the tibial at the ankle contain sensory fibers mediating proprioceptive impulses from muscle as well as other types of impulses. Thus from a purely functional viewpoint there may be a difference in the excitatory and inhibitory influence upon the extensor and flexor half-centers. It might be that the variations in response are due to differences in the intraspinal terminations of the fibers in the different nerves in relation to the half-centers governing flexion and extension at the ankle. This type of reversal might not be present at all in the reflexes at the knee joint. We have not investigated this point.

The rule of reflex response is applicable to the reflexes of the hind-limb at the ankle joint when it is properly qualified. It is subject to variations both on the ipsilateral and contralateral side. To arrive at what is a normal response would necessitate a consideration of the requirements and exigencies of the environment to which the animal would be subjected. There would be as many "normals" as there are changes in the environment to which the animal must react to preserve its well-being in the best manner. These observations confirm completely those of Graham Brown (1912) which led him to an emphasis of the absence of demarcation between extension and flexion.

CONCLUSIONS

Contralateral reflexes have been examined in the muscles acting at the ankle joint in decerebrate, decapitate and spinal animals. The state of neural balance in the spinal centers is a very important factor in determining the type of reflexes present. Reversals have been seen in a great many of these preparations. Weak stimuli usually elicit extension reflexes and stronger ones tend to bring in flexion. Changes in reflex type were found on changing the nerve stimulated. The saphenous nerve gave crossed extension in the anemic decerebrate preparations while the tibial at the ankle and the femoral at times showed flexor involvement either as cocontraction, alternate contraction or pure crossed flexion. Similar reversals were seen in decapitate and spinal animals but in the latter the tibial sometimes gave extension responses and the saphenous flexion. In the decerebrate preparations, rapid faradic stimulation sometimes showed flexor participation in the response while slower stimulation of the same strength gave crossed extension alone. This frequency reversal was not seen in decapitate and only once in spinal animals.

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MOTOR DISTURBANCES CONSEQUENT UPON EXPERIMENTAL LESIONS OF THE CEREBRAL CORTEX

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A number of investigators[1] have observed disturbances in the behavior of the contralateral limbs after experimental injury to parts of the cerebral cortex which give movements of these limbs on electrical stimulation. In all cases, the lesions performed were more or less extensive, involving a considerable portion if not all of the electro-stimulable area. We have believed it interesting to report data[2] constituting a more systematic study of the behavior of an animal's limbs when lesions of different sizes are performed in this part of the cerebral cortex. Accordingly, we have studied the severity of the symptoms in relation to the size or type of lesion performed; we have studied the symptoms also in relation to the species of animal employed. Sixteen guinea pigs, twenty-six cats, and twenty-four dogs were experimented upon.

Three types of lesion were performed: 1, lesions restricted to a motor point of the cerebral cortex determining the movements of an opposite limb; 2, lesions extending beyond a motor point so as to include neighboring motor points determining the movement of the same limb; 3, lesions involving the entire motor area (cruciate gyrus in case of dog or cat) determining the movement of both opposite limbs. In the dogs and cats all three types of lesion were performed; in the guinea pigs the first and second types were performed. After the respective operations, the guinea pigs were studied for a period of five to twelve hours, the cats for a period of six to twelve weeks, the dogs for a period of forty-eight hours.

METHODS. *1. Operative procedure.* The guinea pigs were operated septically, the cats and dogs aseptically. All operations were performed under

[1] Schiff (11), Hitzig (4), Goltz (3), Munk (8), Nothnagel (9), Carville and Duret (1), Ferrier (2), Luciani and Tamburini (5, 6), Schaffer (10), Mott (7), etc.

[2] The data have been collected during the course of experiments performed at the Laboratory of General Physiology, University of Paris, at the Neuro-Surgical Laboratory, Columbia University, and at the Biological Laboratory of the Memorial Hospital, New York City. The data have already been reported by the way of demonstration at the meetings of the Federation of American Societies for Experimental Biology held at Chicago, March 26–29, 1930.

light ether anesthesia. Lesions restricted to a motor point of the cerebral cortex were made by means of a fine cataract knife. The size of the lesion was about $1\frac{1}{2}$ mm. to 2 mm. in diameter. Lesions extending beyond a motor point so as to include neighboring motor points were also made by means of a fine cataract knife. The size of the lesion was about 4 mm. to 6 mm. in diameter. Lesions involving the entire motor area of one side of the cerebral cortex concerned with the movements of both contralateral limbs were made by means of a small gouge. All lesions irrespective of size were made to a depth of about $1\frac{1}{2}$ mm. to 2 mm. Care was taken

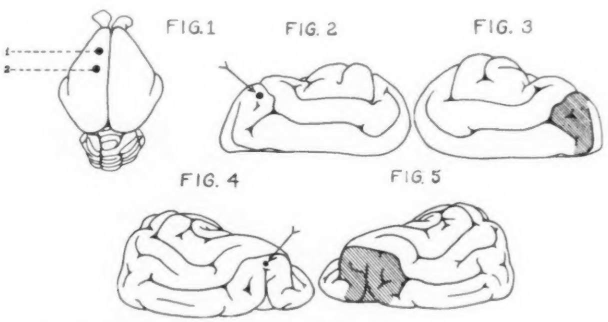

Fig. 1. A guinea-pig's brain in which the cerebro-cortical grey matter was removed from a motor point determining the movement of the right anterior limb and from a motor point determining the movement of the right posterior limb. *1* indicates location of lesion for the anterior limb. *2* indicates location of lesion for the posterior limb.

Fig. 2. A cat's right cerebral hemisphere in which the cortical grey matter was removed from a motor point determining the movement of the left anterior limb; arrow indicates location of lesion.

Figs. 3 and 5. A cat's left cerebral hemisphere (fig. 3) and a dog's right cerebral hemisphere (fig. 5) in each of which the cruciate gyrus was removed; shaded portion in each indicates location and extent of lesion.

Fig. 4. A dog's left cerebral hemisphere in which the cortical grey matter was removed from a motor point determining the movement of the right anterior limb; arrow indicates location of lesion.

to limit the lesions to this depth in order not to carry the ablations into the substantia alba.

2. *Location of motor points.* Liminal galvanic stimuli were employed in locating the motor points determining the movements of the limbs. In all animals, the movements were crossed. The explorations for motor points were made regardless that the animals were under light ether anesthesia.

Results. 1. *Lesions restricted to a motor point determining the movement of a contralateral limb.* In all animals removal of the cortical gray

matter from a motor point determining the movement of a contralateral limb effected the muscle tone and behavior of the limb. The loss of muscle tone and the abnormality in the behavior of the limb were always greater for the guinea pig and cat than for the dog and in each species the effects were always more marked when the lesion involved an anterior limb.

In the guinea pig and cat, the movements of the affected limb were awkward and unsteady. The foot doubled under; more frequently it crumpled. In correcting the defect the animal dragged the foot against the surface of the floor. The foot could be fixed in an abnormal position such as doubled under or crumpled with the toes buckled up. In the cat the above symptoms began to disappear after the first day when the lesion involved a

Fig. 6

Fig. 7

Fig. 6. Fixation of left anterior limb in a cat six weeks after having removed the cerebro-cortical grey matter from a motor point determining the movement of the limb. The cat's right cerebral hemisphere is given in figure 2.

Fig. 7. Fixation (crumpling) of right anterior limb in a dog during the first hours after removal of the cerebro-cortical grey matter from a motor point determining the movement of the limb. The dog's left cerebral hemisphere is given in figure 4.

posterior limb; they began to disappear after two or three days when the lesion involved an anterior limb. The recuperation was always more rapid for the posterior limb. By the end of the fourth, fifth or sixth week it was necessary to study either limb closely in order to detect defects. A certain degree of atonia and unsteadiness remained.

In comparison with the guinea pigs and cats, removal of the gray matter from a motor point of the dog's cerebral cortex effected only slight loss of muscle tone and only slight abnormality in the behavior of the limb. The loss of muscle tone and the abnormalities of behavior were limited in every case to the region of the foot. Though slight and though of the same nature, the symptoms were always more evident for the foot of the anterior limb. During slow locomotion the movement of the affected foot was somewhat awkward and unsteady; occasionally the animal dragged the tips of the

toes against the surface of the floor. At times, it was possible to fix the foot in a crumpling position for a few moments; most of the time the animal resisted fixation.

No abnormalities were noticed when the dog ran or when it ascended a flight of stairs. In descending a flight of stairs, abnormalities were noticed only when the lesion involved the foot of an anterior limb; the foot became unsteady and the animal favored it by leading with the anterior foot of the opposite side.

Twenty-four hours after the operation the abnormalities noted above disappeared in spite of the presence of some loss of muscle tone. The animal was active; it frisked and ran about the laboratory.

2. Lesions extending beyond a motor point so as to include neighboring motor points determining the movement of the same limb: In each species, the loss of muscle tone and other abnormalities described when the lesion was restricted to a single motor point became more pronounced. The abnormalities were always more marked for the guinea pig and cat than for the dog; they were also more marked when an anterior limb was involved.

In the guinea pig the affected limb frequently sprawled forwards or to the side. Rising on all fours from a position of rest was difficult. The animal usually made a series of sprawling movements with the affected limb before establishing itself on all four limbs. Little or no resistance was encountered in fixing the limb in an abnormal position such as extended backwards or to the side with the sole of the foot facing upwards.

In the cat, the atonia and fixation extended beyond the lesion of the foot but not so as to involve the entire limb. During locomotion the foot crumpled and doubled under very frequently. The limb was used very awkwardly, the animal lifting it to a greater height than normal. At times, the anterior limb doubled under in such a manner as to make the animal fall on the side of the limb. In the case of the posterior limb, the animal seldom fell on its side when the foot doubled under. The symptoms were always more marked during the first twenty-four hours following the operation. The recuperation was always more rapid for the posterior limb, the residual symptoms—for five or six weeks after the operation—being always more marked for the anterior limb. For either limb, however, the residual symptoms were always more marked than those described when the lesion was restricted to a single motor point. In the case of the anterior limb, the foot still showed a tendency to double under and drag against the surface of the floor. In the case of the posterior limb, the doubling under was not very marked; the animal rectified the defect as soon as there was an initial crumpling.

In the dog, the symptoms were again limited to the region of the foot. The animal did not resist fixation; the foot could be doubled under quite readily. During slow locomotion, the foot occasionally doubled under.

When sitting or when standing on all fours, the foot also occasionally doubled under. No abnormalities in the behavior of the foot were noticed when the animal ran or ascended a flight of stairs, but when descending a flight of stairs doubling under of the foot was observed. If the lesion involved the foot of an anterior limb, the animal always favored the foot when sitting, standing or when descending a flight of stairs. During locomotion the animal in contrast to the cat did not lift the foot to an abnormal height and when the foot doubled under, the animal did not fall on its side. Turned around in a direction homolateral or contralateral to the lesion, the foot doubled under but again the doubling under failed to cause the animal to fall on its side. Whenever the foot doubled under, the animal

Fig. 8

Fig. 9

Fig. 8. Fixation of guinea-pig's right limbs (anterior and posterior) after removing the cerebro-cortical grey matter from a motor point determining the movement of the anterior limb and from a motor point determining the movement of the posterior limb. Guinea pig's brain is given in figure 1.

Fig. 9. Fixation of a cat's right limbs ten days after removing the contralateral cruciate gyrus. The anterior limb was doubled under while the posterior limb was pulled outward to the side. The cat's left cerebral hemisphere is given in figure 3.

rectified the defect almost immediately, but in so doing it dragged the foot against the floor.

Twenty-four hours after the operation, the above abnormalities in the behavior of the foot diminished considerably, the recuperation being always more rapid for the posterior foot. For either foot (anterior or posterior), the recuperation was always more rapid than the recuperation occurring in the cat. If turned in a direction homolateral to the lesion, the foot displayed an appreciable degree of unsteadiness; if turned in a direction contralateral to the lesion, the foot occasionally doubled under besides showing some unsteadiness. Some loss of muscle tone was still present. When sitting or standing, the foot was in a normal position—

no crumpling or doubling under. If the lesion involved the foot of an anterior limb, there was still some evidence that the animal favored the foot when descending a flight of stairs, and if the descent were made rapidly the foot occasionally doubled under.

3. Lesions involving the entire cruciate gyrus of one side: As already stated above, this type of lesion was performed in cats and dogs. In the cat the lesion severely affected both limbs of the contralateral side. The immediate results were so severe as to make them almost qualitatively different from those effected by the preceding types of lesion. Loss of muscle tone was markedly affected in all sections of each limb. The limbs were typically sprawled out to the side forwards or backwards. Either limb could be fixed as a whole. The animal had difficulty in rising on all fours. When it succeeded, it frequently fell immediately after having gained its standing posture because the affected limbs collapsed, thus causing the animal to fall on the side of these limbs. In spite of their severity, the symptoms were always more marked for the anterior limb.

Considering the size of the lesion, the recuperation was rather rapid. Within the first twenty-four hours after the operation, or shortly thereafter, the animal was often seen walking in a limping fashion for short periods of time. Two to three days later the locomotion began to improve, but the movements were very awkward, more so for the anterior than for the posterior limb. The animal lifted the limbs to an abnormal height.

Three to four weeks after the operation, the animal's locomotion began to approximate the locomotion of the normal animal. Most of the limping disappeared, but an appreciable degree of atonia, unsteadiness and awkwardness of movement persisted in the lower parts of the limbs. Residual symptoms were observed even three months after the operation; they persisted in a mild form and were restricted mainly to the region of the feet. During locomotion slight crumpling of the feet caused the animal to brush the feet against the floor. This disturbance did not occur very frequently; it was always more apparent for the foot of the anterior limb. It was still possible to fix—double under—the foot of either limb, but the fixation was rather difficult, especially for the foot of the posterior limb.

In the dog, the loss of muscle tone affecting the limbs of the opposite side extended beyond the region of the feet but it did not involve either limb in its entirety as was the case in the cat. The abnormalities in the behavior of the limbs, though more marked than those caused by the more restricted types of lesion, were not as severe as those occurring when the cruciate gyrus was removed in the cat. The anterior limb appeared always more affected than the posterior limb. The typical disturbances in the behavior of the limbs were greater unsteadiness and awkwardness in the movements of the feet, frequent doubling under of the feet, ease with which the feet could be fixed, a tendency to lift the foot of the anterior limb to an

abnormal height and a tendency to favor the foot of the same limb when sitting, standing or descending a flight of stairs.

Twenty-four hours after the operation, the atonia and other abnormalities diminished. There was occasional crumpling and doubling under of the feet, the defect causing the animal to brush the feet against the surface of the floor. The animal began to resist fixation, the resistance being always greater for the posterior foot; it was very difficult to fix the posterior foot. If the animal were turned in a direction contralateral to the lesion, the feet, i.e., those affected by the lesion, occasionally crumpled or doubled under. This was more in evidence for the anterior foot. The animal continued to favor the foot of the anterior limb when sitting, standing or descending a flight of stairs.

CONCLUSIONS

According to the data reported we may deduce the following conclusions:

1. In the guinea pig, cat and dog, lesions of the electro-stimulable area (motor area) determining the movements of the contralateral limbs, affect the muscle tone and behavior of the limbs. Loss of muscle tone and motor disturbances occur with lesions varying in size or type from a single motor point to involvement of the entire motor area (cruciate gyrus in case of cat or dog). The effect varies in direct proportion to the size of the lesion and it is always more pronounced for the anterior than for the posterior limb.

2. In the different species of animals experimented upon, the same type of lesion does not produce the same degree of atonia nor the same severity of motor disturbances. A lesion restricted to a motor point produces greater atonia and more pronounced motor disturbances in the guinea pig and cat than in the dog. Likewise, a lesion involving several motor points, or the entire electro-stimulable area produces greater loss of muscle tone and more severe motor disturbances in the cat and guinea pig than in the dog.

3. Given the factor of time to operate, the recuperation for a lesion of a given type is always more rapid for the dog than for the cat; in each of these species the recuperation is always more rapid for the posterior than for the anterior limb. In the cat, loss of muscle tone and motor disturbances caused by lesions restricted to a motor point can be observed even six weeks after performance of the lesions; loss of muscle tone and motor disturbances caused by lesions involving the entire cruciate gyrus can be observed even three months after the operation. In the dog the loss of muscle tone and the motor disturbances caused by lesions restricted to a motor point almost completely disappear within twenty-four hours. The case is almost the same when the lesions involve several motor points of the cerebral cortex. With lesions involving the entire cruciate area a good part of the symptoms (loss of muscle tone and motor disturbances) also disappear within twenty-four hours after the operation.

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THE METABOLISM IN PREGNANCY

IV. The Nitrogen Metabolism[1]

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Some ten years ago the senior author began a study of certain phases of the metabolism in normal pregnancy as a basis for the later investigation of the toxemias, a line of approach that had already yielded concrete results in another field (1). While there was a scattered literature, the earlier lack of accurate chemical methods for the examination of the urine (2), of the blood (3), and of other objective data, had led to contradictory reports and thus failed to establish authoritative criteria. It was patent at the outset that single measurements on isolated cases would fall far short of the significance attaching to continuous studies, both ante- and post-partum, on the same individual. The adoption of this procedure added materially to the labor involved and to the difficulties of performance as the element of the patients' continued coöperation assumed a dominant rôle. The advantages were more than compensatory, however, and the work has been caried out on this basis.

For the purposes of the entire program of investigation, subjects for study were drawn from two independent and sharply differentiated sources. Group I was recruited from the patients reporting to the Prenatal Clinic of the Robinson Memorial, supplemented by a few private cases who volunteered for the study. The women of this group were all living in their own homes under no dietary or other special control, their pregnancies taking place under the usual conditions of the individual habit of life.

Groups II and III, on the other hand, were patients drawn from the inmates of two nursing homes for unmarried mothers. Here the conditions were diametrically opposite, as the patients were under constant dietary and hygienic control—in short, living under uniform standard conditions and following a regimen largely foreign to their normal practice. Further, in this group there was a psychic factor in a proportion of the cases, arising from the conditions of the pregnancy. Normal, healthy individuals were selected initially and only those retained who maintained this condition

[1] Presented in part before the American Physiological Society, Chicago, March, 1930.

throughout the period of study. The elimination of those who developed apparent abnormality, and the defection of the much larger group whose interest waned as the study progressed, has limited in a most material way the number of completed cases. Considerably more than two hundred cases were partially studied to yield the series of seventy-seven that forms the basis of these reports. The actual composition of the group and certain descriptive details can be most compactly presented in tabular form.

The only point of remark is the large number of young mothers, a fact, naturally, depending on the source from which so large a part of the group was drawn. The other figures require no comment.

TABLE 1
Composition of series

DATUM	GROUP I	GROUP II	GROUP III	TOTAL OR AVERAGE
	Number of cases			
	25	21	31	77
Age:				
High (years)	37	40	28	40
Low (years)	18	13	14	13
Average (years)	28	18	19	22
Parity:				
High	6	2	2	
Low	1	1	1	
Average	2.4	1+	1+	
Weeks of study:				
Ante-partum:				
High	34	13	25	34
Low	11	4	6	4
Average	21	8	14	15
Post-partum:				
High	10	10	30	
Low	1	2	5	
Average	2	6	14	8

The present paper will be devoted to certain aspects of the protein metabolism, primarily as estimated by the several levels of blood and urine nitrogen. With the exception of that for total nitrogen, for which the standard Gunning modification of the Kjehldahl (5) was used and for uric acid, in which the Benedict-Franke (6,7) procedures were followed all determinations were made by the methods of Folin and his associates (8).

Urine examination: Only complete 24-hour urine collections were studied, and these were collected over toluene in the usual manner and were kept in as cool a place as possible to lessen urea change to ammonia. The

fluctuations in the weekly output, both for the volume of the urine and the amount of total nitrogen, are given in the next table (table 2).

The relative increase in urine volume in pregnancy so frequently reported is well exemplified by these figures. How far this derives from an intrinsic excretory mechanism engendered by gravidity, and how far it is the result of the common advice to drink plenty of water during pregnancy, may be open to question. After delivery the volume falls significantly but the lactating mother is eliminating an appreciable quantity of water through

TABLE 2

Weekly variation in elimination

TIME	AVERAGE URINE VOLUME	AVERAGE TOTAL NITROGEN
weeks	*cc.*	*grams*
Over 25	1,450	
24–21	1,630	9.07
20–17	1,750	8.29
16–13	1,630	8.00
12-9	1,580	8.11
8–5	1,720	7.94
4	1,840	8.14
3	1,780	9.64
2	1,690	8.02
1	1,720	6.73
Average (weighted)......	1,660	8.03
	Delivery	
2	1,350	(8.72)*
3	1,300	7.79
4	1,380	9.46
5–8	1,370	7.68
9–12	1,170	7.12
13–16	1,160	8.42
Over 16	1,230	8.56
Average (weighted)......	1,260	8.04

* Omitted from average. Number of observations small.

the mammary glands. In any case the average elimination post-partum is still somewhat above the average figure for women in a state of sexual rest, and that in spite of an unusual extra water output through other channels.

It has long been recognized that the pregnant woman stores protein and that in amounts far in excess of the requirements of the fetus.

The weekly fluctuations are no more than might be anticipated in so large a group of individuals, all of whom were free to choose the amounts

of food ingested and one-third of the group, further, without any form of dietary control. While the elimination ante-partum is slightly lower than that recorded by Hasselbalch (9), the differences are far less than from a series of normals collated by one of us (10). These may be briefly listed as they bear directly on the thesis.

Folin's high figures are undoubtedly influenced by the composition of the standard dietary followed by his subjects. The other figures show fair correlation. In all of these series there were a number of males and the nitrogen elimination per kilogram of body weight of the young, healthy male is definitely superior (roughly 6:5) to that of the equivalent female. A series of the latter shows an average of 9.4 grams daily (unpublished data A. W. R.). Finally, a group of 200 independent observations made on normal, healthy pregnant women—not under institutional care—gave an average value of 8.22 grams of nitrogen for the daily output. In this latter series the average weight one week ante-partum was 66.1 kilograms, while the group on which the present thesis is based showed an average

TABLE 2-a

Nitrogen elimination

AUTHOR	NUMBER OF SUBJECTS	TOTAL NITROGEN (AVERAGE)
Folin (2)	30	16.00
Matthews (11)	25	11.30
Long and Gephart (12)	201	10.08
Rowe and Proctor (10)	107	10.91

weight of 66.3 kilograms. The present figures may be regarded as representative of normal pregnant women in this part of the world. Further, there can be no question as to the storage of significant amounts of protein, as has been pointed out even more certainly by others.

The maintenance, in the post-partum period, of the same value for the average nitrogen level is in some measure fortuitous. During this latter period due allowance must be made for the nitrogen eliminated in the milk, and it is probably no more than coincidence that the urine fraction remains apparently constant. Another factor for consideration in producing a lower nitrogen output lies in the lower body weight, the average for the series taken between three and four weeks after confinement being 8.9 kilograms or about 13 per cent less. The amniotic fluid is but a small portion of this loss. Again these figures, presumptively at least, fall short of those of Hasselbalch (l.c.) but by comparison with the independent data given above may be regarded as characteristic.

One other point may be touched upon before taking up the nitrogen

partition. The average specific gravity of the urine ante-partum was 1.0170 and was 1.0187 after delivery. This increase, post-partum, in large measure compensates for the lower volume, particularly as the expulsion of the fetus lowers weight (active protoplasmic mass) and relatively lessens the general metabolism.

The data for the nitrogen partition have been collected in the next table. For purposes of comparison these control series have been added i.e., *a*, series of the senior author (8), already referred to, combined with an unpublished series from a group of normal young women in a state of sexual rest, and *b*, a small series of normal pregnant women independently and very thoroughly studied in connection with another investigation.

As might be anticipated from other reports, the urea percentage is below the normal but this is not solely due to an increase in the ammonia. True, the latter is above the normal level and on a percentage basis by a really

TABLE 3
Nitrogen partition

NITROGEN	PARTUM		CONTROLS	
	Ante-	Post-	Normals	Pregnant
	per cent	*per cent*	*per cent*	*per cent*
Urea	79.6	80.8	85.0	80.6
Uric acid	2.8	1.9	2.1	2.1
Ammonia	5.0	5.1	3.4	4.6
Creatinin	4.7	4.2	4.6	4.6
Residual	7.9	8.0	4.9	8.1
Per cent over 9 per cent	36.5	33.3	0	43.7

significant amount. Our values fall definitely short of certain of the high levels reported in the literature but all of the patients in this series were maintained in good nutritional equilibrium and inanition acidosis played no part. It is interesting that the ammonia level is maintained in the post-partum period. Speculation might assign this to a loss of fixed alkali from the blood to supply certain of the minerals of the milk. The fact that potassium and not sodium is the principal milk base offers no direct support for such an hypothesis. None the less lactation introduces a variant in the normal course of metabolism, many of the effects of which have not been thoroughly studied. The residual nitrogen fraction, while within conventional normal limits, shows a distinct upward tendency, and in about one-third of the urines examined is absolutely above the somewhat elastic limits assigned to the norm. The amino-acid content is known to be increased, and so-called polypeptid nitrogen is said to be present in pregnancy. To these, in the later stages at least, must be added creatin;

not impossibly, the undetermined fractions of the residual portion (10) play a part. Uric acid is high in the ante-partum period but drops to a normal level after delivery. While creatinin is largely endogenous and so should approach a constant under all conditions save that of direct feeding, there is apparently a difference of some 40 mgm. of nitrogen or 0.1 gram of creatinin between the two periods. The immediate explanation is not forthcoming as the normal errors of measurement should be absorbed in so long a series.

To summarize, the nitrogen elimination during pregnancy is lowered but is still at a level superior to that of maintenance. Further, the urea percentage diminishes, the major portion of the difference being absorbed by the ammonia and residual nitrogen fractions. Uric acid is also increased. In the first few months after delivery the same general picture persists, the uric acid alone subsiding promptly to a more normal level.

Blood. As the nitrogenous constituents of the urine derive from those in the blood, the concentrations in the latter medium are complementary to any study of the former. The processes of the kidney, however, are highly selective and neither absolute nor relative amounts show any marked degree of uniform correlation. Schematically and using conventionally normal figures, the several relationships are as follows:

TABLE 4

Comparison of blood and urine nitrogen

	CONCENTRATION		PARTITION	
	Urine	Blood	Urine	Blood
	mgm. per 100 cc.	*mgm. per 100 cc.*	*per cent*	*per cent*
Total nitrogen	1,100	33		
Urea nitrogen	935	16	85	48.5
Uric acid nitrogen	22	1.2	2	3.5
Ammonia nitrogen	39	0	3.5	0
Creatinin nitrogen	50	0.6	4.5	2.0
Residual nitrogen	54	15.2	5.0	46.0

While there is no approach to parity in any of the absolute values, the three major differences in the partition are shown by the urea, ammonia, and residual fractions. That the ammonia should present an exception is to be expected as it is primarily the end result of a defense mechanism located in the kidney itself and regulated by the acid base equilibrium and not by the protein metabolism. The absolute blood levels here are based upon the data from individuals on a liberal but not excessive protein diet. Experience in this laboratory has shown that the blood concentrations are somewhat influenced by the level of the protein metabolism but in far less degree than are those of the urine. This is to be expected

as the urine content is an end result, a collection of excreted materials, while the blood levels are determined by several kinetic processes operating to maintain a constancy in the concentration of each chemical entity.

The actual findings with the present series are given in the next table.

The several indications may be briefly reviewed. As has been noted by many others, the non-protein nitrogen is definitely depressed during the period of pregnancy. This is shown both by the subjects of the study and the group of pregnant controls in contra-distinction to the levels post-partum and those of the group of normal young women in a state of sexual rest.

The urea values show the same general relationships and are entirely consistent with each other if regarded independently. Taken in connection with the amounts of non-protein nitrogen, however, a serious question arises. In the literature of the last ten years have appeared a number of records dealing with the ratio of urea to non-protein nitrogen in the blood

TABLE 5

Blood chemistry

	PARTUM		CONTROLS	
	Ante-	Post-	Pregnant	Not pregnant
Non-protein nitrogen	25.0	32.0	27.0	31.0
Urea nitrogen	12.0	16.0	13.0	14.0
Uric acid	3.3	3.7	3.2	3.5
Creatinin	1.5	1.5	1.5	1.5
Residual nitrogen	11.3	14.2	12.3	15.2

of pregnant women. The reported findings are by no means uniform and vary from the one extreme of definite depression of urea nitrogen in normal pregnancy through depression only in pathological cases, to the other extreme of a wholly normal ratio. In a recent paper by Denis and associates (13), the average percentage during pregnancy (average of 2 samples each from 162 subjects) is 33 per cent, in a smaller group (55) post-partum the level is 43 per cent, and in four non-pregnant women 46 per cent. They state further that where monthly samples could be secured it was possible to trace a gradual lowering of the ratio as gestation progressed. These findings are significantly at variance with our own, as the accompanying table will show.

The interval fluctuations here are no more than are to be expected where rounded numbers are used. A weighted average for the individual percentages is 47; a like procedure with the post-partum data yields 49 per cent. Even raising the non-protein by one milligram and lowering the urea nitrogen by the same amount to over compensate for the rounded

numbers, yields only 42 per cent for the ratio, a value far superior to that quoted above. Dilution of the blood, known to exist in pregnancy, can not be the explanation as it would affect both factors alike.

A group of patients, examined independently by Dr. R. S. Hunt, to whose courtesy we owe this datum, shows a consistent average of 44 per cent.

While the more recent methods for uric acid give lower values, the older method first adopted (Benedict-Franke from 1922) has been retained throughout in order that the data should be comparable throughout the series. Uric acid shows an equivalent upward trend in the post-partum period; all values are well within normal limits as defined by the method used; creatinin remains at a constant level. The residual nitrogen values are extremely interesting, more by way of suggestion than of definition. The absolutely low level ante-partum is inconsistent with the suggestion that the low urea ratio depends upon an increase in this fraction.

TABLE 6

Urea percentage

TIME	N P N	UREA N	PER CENT
weeks			
24–21	25	11	44
20–17	25	12	48
16–13	24	11	46
12–9	24	11	46
8–5	25	12	48
4	25	12	48
3	25	12	48
2	26	12	46
1	26	13	50

It will be remembered that the residual nitrogen in the urine presumably deriving from this fraction showed an upward tendency, a condition, as has been noted above, which is in no sense incompatible with a low blood level. Unfortunately amino-acid contents were not determined, an omission now being corrected.

To summarize briefly, non-protein, urea, and residual nitrogen all exhibit lower absolute levels in pregnant women than in those at an equal nutritional level when in a state of sexual rest.

SUMMARY

The levels of certain of the nitrogen-containing constituents of the blood and urine during pregnancy have been determined by the repeated investigation of a series of healthy, well-nourished women throughout the period of gestation. Similar studies have also been conducted after delivery. The variations induced by gravidity have been briefly discussed.

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THE ACTION OF ABDOMINAL SYMPATHECTOMY ON THE GROWTH OF THE ALBINO RAT AND THE WEIGHT OF THE GENITAL ORGANS

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The influence of sympathectomy on growth has already been studied by several investigators, in the cat (Cannon and collaborators, Wright and Harris) and in the rabbit (Bidder, Wright and Harris, René Simon). After having developed a simple technique—to be reported in another paper—for removal of the two abdominal sympathetic chains of the rat, we thought that it would be of some interest to operate some young rats, and observe their growth comparatively with animals of the same litter kept as controls; for, after the extensive work of Donaldson, the rat may be considered as the best standard animal in which even small differences can be detected.

The animals used were derived from the Wistar colony. Healthy litters of 4 or 5 young of the same sex were chosen. When the weight of these rats reached 60 to 75 grams, bilateral abdominal sympathectomy from the pelvic ganglia to a level situated somewhat above the level of the renal vessels was performed under ether anesthesia. In one series (table 2) we also removed the right adrenal gland and denervated the other by section of the splanchnic nerve and excision of the cœliac ganglion. Sham operation was performed on the control rats. None of the animals died. Operated and control rats were kept in the same conditions of nutrition and environment. No sexual indulgence was allowed.

As regards appearance and general behavior, sympathectomized animals were indistinguishable from the normal. About three months after the operation, the rats were killed by ether. We noticed several times (namely, in rats 15, 28 and 17) a failure of the hair to stand on the lower part of the back as a result of ether asphyxia, while the hair on the head and thorax was fully erect. Furthermore, careful post-mortem examination showed that the operation had been, except in one case, complete (in that case, rat 21, the third left lumbar ganglion had been left intact), the sympathetic

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chains having been removed at least to the second lumbar ganglion. Regenerated fibres going from the level of the section in the lumbar muscles

TABLE 1

Rats born December 22, 1929. Abdominal sympathectomy and sham operations performed February 5, 1930. Animals killed May 8, 1930

NUMBER	BODY LENGTH	TAIL LENGTH	BODY WEIGHT	KIDNEYS	TESTES	SEMINAL VESICLES AND PROSTATE	EPIDIDYMIDES	DRY FEMURS
	cm.	*per cent*	*grams*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
13: abd. sympath.	19.7	90.9	184	0.750	1.32	0.792	0.413	0.397
14: abd. sympath.	20.4	91.3	208	0.744	1.01	0.746	0.390	0.384
15: control	20.8	92.1	222	0.691	1.18	0.682	0.340	0.395
16: control	19.5	91.5	180	0.763	1.17	0.703	0.379	0.387
19: control	19.8	91.0	186	0.731	1.20	0.669	0.376	0.361

TABLE 2

Rats born December 27, 1929. Abdominal sympathectomy with adrenal inactivation, and sham operations performed February 7, 1930. Animals killed May 9, 1930

NUMBER	BODY LENGTH	TAIL LENGTH	BODY WEIGHT	KIDNEYS	TESTES	SEMINAL VESICLES AND PROSTATE	EPIDIDYMIDES	DRY FEMURS
	cm.	*per cent*	*grams*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
28: operated	19.2	89.1	186	0.775	1.31	0.728	0.443	0.375
29: operated	19.5	90.2	214	0.752	1.04	0.808	0.420	0.328
25: control	19.3	91.0	190	0.768	1.21	0.668	0.405	0.358
26: control	19.6	90.0	197	0.670	1.13	0.670	0.405	0.360
27: control	19.8	92.5	214	0.724	1.06	0.715	0.364	0.352

TABLE 3

Rats born December 22, 1929. Abdominal sympathectomy and sham operations performed February 13, 1930. Animals killed May 20, 1930

NUMBER	BODY LENGTH	TAIL LENGTH	BODY WEIGHT	KIDNEYS	OVARIES	UTERUS AND TUBES	DRY FEMURS
	cm.	*per cent*	*grams*	*per cent*	*per cent*	*per cent*	*per cent*
17: abdom. sympath.	18.7	91.5	164	0.927	0.0488	0.464	0.418
21: abdom. sympath.	19.0	92.1	169	0.908	0.0520	0.379	0.445
20: control	18.4	94.0	152	0.838	0.0353	0.175*	0.452
18: control	17.7	91.0	132	0.910	0.0417	0.326	0.425

* The left tube was atrophied.

and in the mesocolon were found in most cases, and the left adrenal of rats 28 and 29 was no longer denervated.

Thus, the hind limbs were certainly without sympathetic innervation, while the genital organs were probably only partially denervated (fibres coming from the first lumbar ganglion were left intact, and regeneration probably occurred). Dissection and weighing of organs were carried out according to the technique described by Donaldson (1924). The results of our measurements are given in tables 1, 2 and 3; the weight of the different organs is expressed in percent of body weight, and the tail length in percent of body length, in order to facilitate the comparison between operated animals and controls.

The total weight of the hind limbs was also recorded and in every case found within the normal range. No abnormal distribution of fat was noticed. Obviously, there was no difference in body weight or in body length between operated and control rats. The average tail length was 90.8 per cent of body length in operated animals and 91.6 per cent in the controls. The average weight of the femurs was 0.391 per cent of body weight in operated animals, and 0.386 per cent in controls. Abdominal sympathectomy had clearly not affected the growth of the tail or the hind limbs of our rats.

Our work confirms that of Cannon and collaborators (1929) who removed the sympathetic chain on one side from two kittens and compared the operated side with the other. Wright and Harris (1929) also observed no difference after unilateral sympathectomy in the cat. In the rabbit, however, Bidder (1874) and Wright and Harris (1929) observed an increased growth of the ear after resection of the corresponding cervical sympathetic chain; but René Simon (1930) lately was unable to observe any difference in the ear length of 18 rabbits from which the cervical sympathetic with the stellate had been removed on one side. (It should be noted that the animals used by Wright and Harris were operated much younger.) According to Wright and Harris, the rabbit's ear is a peculiar case, and the effect observed was due, not to the increased blood supply, but to the increased temperature. At any rate, it may be concluded that sympathectomy does not affect the growth of the limbs or bones.

In opposition to these negative results, our tables show that the weight of the epididymides and of the seminal vesicles and prostate is, on the average, 10 per cent higher in the operated animals (other organs like the kidneys and the testicles do not show this difference). This fact is in accordance with other observations on the guinea pig, to be reported in another paper; it is due to a lack of expulsion of the secretion.

The weight of the ovaries and genital tracts of the two sympathectomized females is also much above the controls and above Jackson's data reported in Donaldson's monograph. These organs were macroscopically normal, and we are unable to explain this difference of weight.

CONCLUSIONS

1. Removal of the two abdominal chains from young albino rats has no influence on the later growth of the tail and hind limbs.

2. An increased weight of epididymides, seminal vesicles and prostate was observed in four sympathectomized males; the weight of the ovaries, uterus and tubes in two sympathectomized females was also much above the normal.

It is a pleasure to thank Professor Cannon for his suggestions and kind advice.

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THE HEART RATE AFTER SYMPATHECTOMY AND VAGOTOMY AND THE BLOOD SUGAR AS AFFECTED BY POSTERIOR HYPOPHYSEAL EXTRACTS (PITRESSIN AND PITOCIN)

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The present investigation had as its primary object a comparison of the action of post-pituitary extracts in sympathectomized and normal animals. It was subsequently decided to include observations on animals in which one or other set of the heart nerves had been removed, so that our experiments are, to some extent, an analysis of the manner in which the characteristic changes in heart rate are brought about.

Most of the experiments were on surviving, unanesthetized cats. Several acute experiments were carried out for reasons stated below. The drugs used—pitressin and pitocin (Parke, Davis)—were given intravenously by way of the marginal ear vein. This vessel can be entered easily with a no. 28-gauge needle even in the unanesthetized animal, and the uncertainties of extra-vascular administration avoided. The amounts injected per animal varied from 0.075 cc. to 0.2 cc. of the commercial preparations (containing, in the case of pitressin, 1.5 to 4 pressor units, and, in the case of pitocin, 0.75 to 2.0 oxytocin units). The heart rate was counted by means of a stethoscope.

I. ACTION ON HEART RATE. A. *Experiments on normal cats.* Six normal cats received pitressin in the amounts stated in table 1. Care was taken to keep the animals quiet not only during the experiments, but for a time preceding the injection, so as to start with a heart rate as slow as possible. In every instance, the injection induced a profound slowing. The decrease in rate began almost immediately and reached its maximum in 1 to 3 minutes (table 1). This effect was transient, however, the original rate being restored in 5 to 10 minutes. We have never observed any secondary changes.

Pitocin was administered to 4 animals. No definite or marked effects on heart rate or on the general behavior of the animals were noted (table 2). These observations are in agreement with the findings of Gruber (1929).

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TABLE 1

Pitressin and heart rate in normal cats

NUMBER	AMOUNT OF PITRESSIN INJECTED	RESTING HEART RATE	MAXIMUM SLOWING TO
	cc.		
301	0.2	114	82
302	0.2	112	76
300	0.2	114	60
442	0.05	94	68
303	0.2	104	70
297	0.2	156	96

TABLE 2

Pitocin and heart rate in normal cats

NUMBER	AMOUNT OF PITOCIN INJECTED	RESTING HEART RATE	EFFECT OF INJECTION
	cc.		
303	0.15	100	No change
302	0.2	120	Slowing to 112
299	0.2	168	Slowing to 148
304	0.2	116	Slowing to 104

TABLE 3

Pitressin and heart rate in sympathectomized cats with vagus innervation of the heart intact

NUMBER	AMOUNT OF PITRESSIN INJECTED	RESTING HEART RATE	MAXIMUM SLOWING TO
	cc.		
295	0.2	106	70
494	0.2	108	62

TABLE 4

Pitressin and heart rate in sympathectomized animals with vagus innervation of the heart absent

SOON AFTER DENERVATION OF THE HEART				MORE THAN TWO MONTHS AFTER DENERVATION OF THE HEART			
Number	Amount injected	Resting heart rate	Maximum acceleration to	Number	Amount injected	Resting heart rate	Change in heart rate
	cc.				*cc.*		
286	0.2	126	160	294	0.1	126	Acceleration to 138
294	0.075	110	148	294	0.1	124	Acceleration to 134
287	0.13	118	188	286	0.1	160	Prompt slowing to 100
288	0.15	92	106				

B. *Experiments on sympathectomized cats.* The sympathectomy operations were performed in accordance with the technique described by Cannon, Newton, Bright, Menkin and Moore (1929). The animals, at the time of use, were in good condition, having completely recovered from the acute effects of the operations.

1. *Cats with vagus innervation intact:* In two animals (cats 494, 295—table 3) both sympathetic chains were removed, while the vagus innervation to the heart was left intact. In both cases pitressin caused a reduction in heart rate, very similar to that noted in normal animals. Here, however, the duration of the slowing exceeded that observed in normal animals, the basal level not being restored for about 20 minutes.

2. *Cats with vagus innervation excluded:* In other animals (cats 294, 286, 287, 288—table 4) not only the sympathetic chains but the vagus branches to the heart were removed. Under these conditions the effects of pitressin were strikingly different from those previously noted. Three animals, tested on several occasions two weeks to two months after the denervation, showed a marked increase in heart rate; the fourth gave neither acceleration nor slowing. These results indicated that, in the absence of the central connections of the cardiac nerves—particularly the vagi—slowing of the heart after pitressin does not occur. The possible cause of the acceleration will be considered later.

Another effect of pitressin upon the completely denervated heart was disturbance in rhythm, with extra systoles and skipped beats. These changes may persist as long as 30 minutes. They are not specific with pitressin, however, for they also occur after adrenalin and after insulin.

One to two months later, while two of the cats were still in excellent condition, we attempted to repeat the experiments with pitressin. To our surprise, the acceleration was no longer demonstrable. Cat 294 showed practically no change in heart rate, whereas previously a marked increase had occurred. Cat 286 gave a definite slowing. This brought up the possibility of there having occurred a reëstablishment of vagus connections in at least one of these animals within two to three months after the operation. Evidence that vagus re-growth may occur even after so short an interval will be reported in another communication.

We therefore decided to reëxamine the action of pitressin upon the recently denervated heart, and carried out four acute experiments for this purpose. A typical protocol follows:

Normal cat 63. Light ether anesthesia. Artificial respiration

9:50 a.m.	Right vagus nerve cut in neck
9:55 a.m.	Left vagus nerve cut in neck
9:58 a.m.	Heart rate 256 per minute
10:11 a.m.	Heart rate 212 per minute
10:11 a.m.	0.2 cc. pitressin injected intravenously

10:12 a.m. Heart rate 200
10:14 a.m. Heart rate 196
10:15 a.m. Heart rate 190
10:28 a.m. Heart rate 202
10:30 to 10:50 a.m. Bilateral upper thoracic sympathectomy
11:04 a.m. Heart rate 144
11:07 to 11:09 a.m. Heart rate 152–148
11:10 am. 0.2 cc. pitressin injected intravenously
11:10½ a.m. Heart rate 152
11:11 a.m. Heart rate 156
11:12 a.m. Heart rate 148
11:13 a.m. Heart rate 148
11:15 a.m. Heart rate 150

All four experiments gave similar results. After both vagus nerves had been removed, the heart rate was rapid; in this condition pitressin caused a definite, though not marked, slowing. Subsequent excision of the cardiac accelerators was followed by a considerable reduction in heart rate—much more, it will be noted, than that previously caused by the drug. At this time, injection of pitressin had practically no effect. It is therefore evident that in the recently denervated heart pitressin causes no slowing. It follows that the slowing of the heart rate that occurs in the intact animal is due to some action upon the cardiac nerves, and involves not only the vagus but also to some extent the sympathetics. The amount of slowing that may occur after double vagotomy—while the cardiac accelerator fibres are present—is indicated in the following experiments.

TABLE 5

Pitressin and heart rate in vagotomized cats

NUMBER	AMOUNT OF PITRESSIN INJECTED	RESTING HEART RATE	MAXIMUM SLOWING TO
	cc.		
493	0.2	190	158
291	0.2	200	166
292	0.2	190	144
292	0.2	196	152

C. *Experiments in surviving vagotomized cats.* In three animals the left vagus was sectioned in the neck, while the right vagus nerve was sectioned in the chest below the recurrent laryngeal branch. The animals were allowed to recover, and remained in good condition for a time. The results of four experiments with pitressin are shown in table 5. The heart rate at the start of the experiments was always very rapid, regardless of the state of the animal. In all instances the injection of pitressin was followed by a certain degree of slowing. The reduction in rate came on more or less gradually, the slowest rate being observed 15 to 30 minutes after the in-

jection. The original "basal" rate did not return for about 1 hour. This reduction in rate after vagotomy is more prolonged than any other observed effect of pitressin upon the heart. It is to be noted, however, that even when the most extensive slowing occurred, the rate was still much above that considered basal in a normal animal.

The effect of pitressin upon the rate of the heart under the different conditions is illustrated in figure 1.

II. Effect on blood sugar. In 6 normal and 5 sympathectomized cats, the action of pitressin on blood sugar was investigated. The effect of

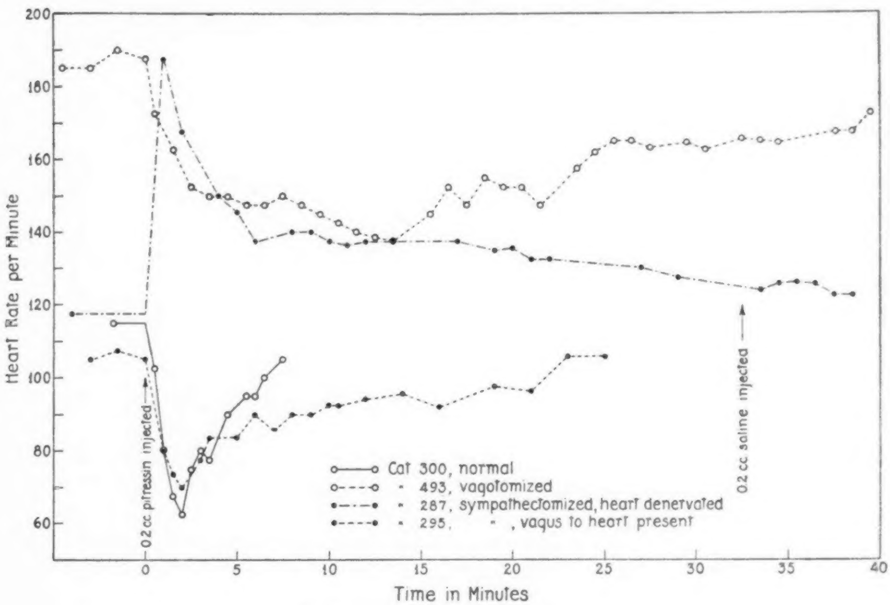

Fig. 1. Pitressin and heart rate

pitocin was observed in 2 normal animals. Blood was removed from one of the ear veins, and the sugar determinations made by the Hagedorn-Jensen (1922) method.

A. *Normal animals.* Both pitressin and pitocin cause a marked increase in blood-sugar concentration. In figure 2 are shown the results of six experiments with pitressin. The rise in blood-sugar content begins almost immediately after the intravenous injection, the peak being reached in 3 to 10 minutes. Both the extent of the increase as well as its duration may vary in different animals; but it is noteworthy that the original blood-sugar may not be regained for two hours.

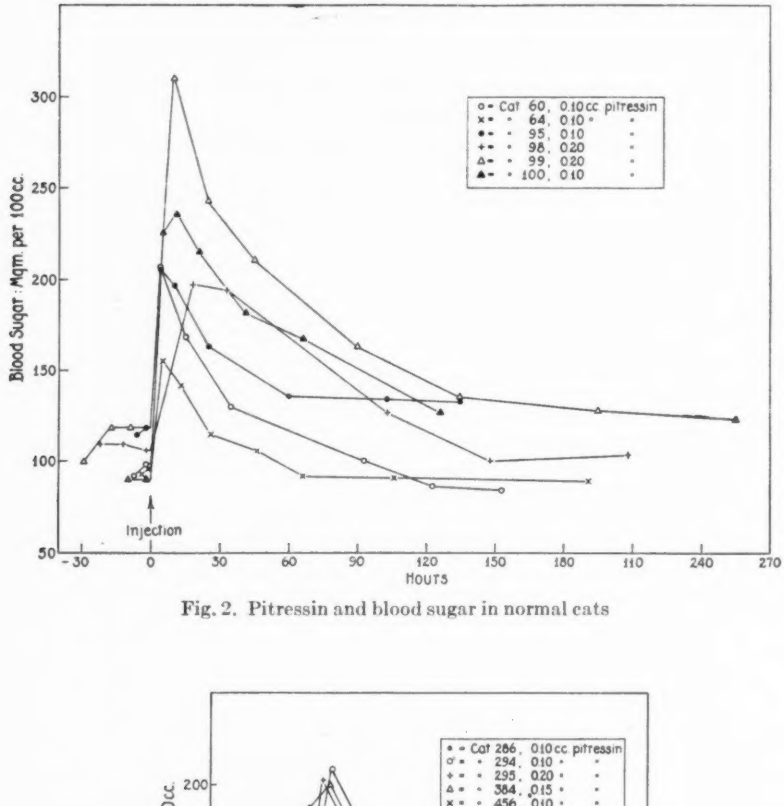

Fig. 2. Pitressin and blood sugar in normal cats

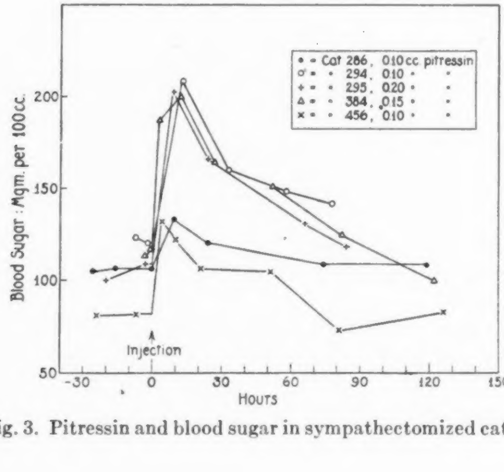

Fig. 3. Pitressin and blood sugar in sympathectomized cats

B. *Sympathectomized animals.* The results of five experiments in sympathectomized cats are shown in figure 3. In these animals, too, a marked glycemia resulted. While the degree of the reaction—as well as its duration—seems to be somewhat less than in normal animals, we feel that our data are insufficient to enable us to make a definite distinction. There is no doubt that the increase in blood-sugar concentration occurs just as soon after the injection as in normal animals, and that the hyperglycemia in some cases may persist for about as long a time.

III. Effect on respiration. Sympathectomized as well as normal animals showed marked respiratory changes after the pitressin injection. Polypnea and dyspnea were common, the respiratory rate being often increased to 110 per minute, and becoming for a time irregular. These effects soon passed off. Some animals for a brief time after the injection showed acute distress; in one instance tonic and clonic convulsions set in. It may be mentioned here that dogs usually show a much more intense reaction—thus two animals died a few minutes after receiving 0.2 cc. of pitressin intravenously.

Discussion. The result of the present investigation must be considered in the light of previous knowledge of the action of posterior hypophyseal extracts. In 1898 Howell observed that injection of the posterior pituitary body not only increased blood pressure, but slowed the cardiac rhythm. Slowing of the pulse has been found by many subsequent investigators; in the unanesthetized animal it has been observed by Kolls and Geiling (1924) and in man by Bell (1919) and Sachs (1924). The recent paper of Gruber (1929) mentions a reduction in pulse rate after pitressin.

The manner in which this slowing is brought about has never been entirely ascertained. The generally accepted explanation involves two actions, the first being stimulation of the cardio-inhibitory center, the other, a direct action upon the myocardium (Wiggers, 1911). According to Wiggers, "either both, or neither, of these actions may follow the injections of pituitary extract—if both effects are present, vagus section will modify but not abolish the slowing." Our results indicate that the slowing of the heart rate after pitressin is entirely due to an action upon the extra-cardiac connections of the heart nerves. Both sets of nerves seem to be concerned in this slowing. Undoubtedly the most pronounced effect is mediated by the vagi. But the facts that even after bilateral vagotomy pitressin still slows the heart rate, and that this effect is abolished by removal of the accelerator nerves, indicate that the latter are concerned in the slowing after the vagi have been cut. It seems to be clearly established that the rate of the completely denervated heart (within the animal) is not reduced by pitressin. This finding is contrary to the observations of several workers who found slowing of the isolated (perfused) heart with posterior pituitary extracts (Hedbom, 1899; Cleghorn, 1899). An increased heart rate has,

however, been reported by Einis (1913). In any case, it is very difficult to compare observations on the denervated heart within the animal with those obtained on the excised organ. The marked acceleration observed by us in the surviving, unanesthetized animal soon after sympathectomy and heart denervation cannot be correlated with any previous work, except that of Einis.

On blood sugar, the augmenting action of posterior pituitary extracts has been known for many years. Our observations in normal cats are in agreement with the recently published work of Nitzescu and Benetato (1930) in dogs and rabbits—to the effect that both pitressin and pitocin, given intravenously, induce a marked and comparatively long-lasting hyperglycemia. The promptness with which this hyperglycemia appears implies an immediate discharge of sugar into the blood stream. The occurrence of the glycemic reaction in sympathectomized animals indicates that the sympathetic nervous system is not essential in this release of sugar. To what extent the response is modified in these animals we are unable, with our data, to state.

CONCLUSIONS

1. In normal cats pitressin, injected intravenously, causes a prompt reduction in heart rate (confirming Gruber's observations). Pitocin has little effect.
2. In surviving vagotomized cats, pitressin reduces the heart rate, but to a level still far above the average basal rate for a normal animal.
3. In cats surviving excision of both sympathetic chains, the action of pitressin upon heart rate is dependent upon the presence or the absence of (central) vagus innervation to the heart. Slowing occurs when the vagi are present; marked acceleration when these nerves have been sectioned.
4. Both pitressin and pitocin, given intravenously, cause a prompt rise in blood-sugar concentration. The hyperglycemia may last as long as two hours.
5. In sympathectomized cats the glycemic effect of pitressin is somewhat less than in normal animals, but not markedly so.

We wish to express our thanks to Prof. W. B. Cannon for suggesting this problem.

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THE ACTION OF PARATHYROID EXTRACT IN SYMPATHECTOMIZED ANIMALS

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The operative procedure elaborated by Cannon and collaborators (1929) makes it possible to remove practically the entire sympathetic system in mammals. Such animals may survive the operation for an indefinite period, and remain apparently in good condition. Among the many interesting questions that arise in regard to these animals is whether the working of the endocrine organs is affected by the absence of sympathetic innervation. Likewise, it is of interest to test the action of endocrine extracts in such preparations. The experiments to be reported here were concerned with a comparison of the action of parathyroid extract (Parathormone, Lilly) in sympathectomized and in normal cats, and in dogs. It has been established by numerous investigators that the parathyroid hormone plays an important rôle in the regulation of calcium metabolism. As long as the manner in which the characteristic effect is brought about remains unknown, such observations as are reported below may have some interest.

I. *Observations in normal cats.* Inasmuch as no detailed description of the effects of parathyroid extract upon normal cats seems to have been made, several preliminary experiments were carried out for this purpose. The cats were in good condition at the time of use, and had been kept on a diet of canned salmon, boiled meat, bread and milk. Blood for calcium determinations was removed in 6 to 7 cc. quantities from one of the marginal ear veins. The Clark-Collip (1925) technique for calcium measurements was employed throughout. Six normal cats received Parathormone subcutaneously, in varying amounts. Single injections were made in some instances, repeated injections in others.

The results are shown in table 1. Single injections of 5 to 8 units of the regular commercial extract per kilogram, had little effect upon the blood calcium in 7 to 8 hours. Massive doses given in a relatively long period had a more marked effect; thus, 20 units per kilogram administered over a period of 48 hours induced in cat 100 a hypercalcemia of 17.9 mgm. per

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100 cc. The results of such injections are illustrated in figure 1. Toxic symptoms—polypnea, vomiting, muscular weakness, incoördination—were observed in one cat (95, table 1). This animal received nearly 60 units in the course of 48 hours. Once manifested, the symptoms never entirely disappeared, and the animal died 3 days later after one or two periods of partial recovery. Shortly before the time of death the calcium level had returned to near the basal; apparently death was not due to the increased blood calcium. We never observed slowing of heart rate in any of our dogs or cats, and thus are unable to confirm the findings of Edwards and Page (1926) on dogs. Usually an increased heart rate was noted in our experi-

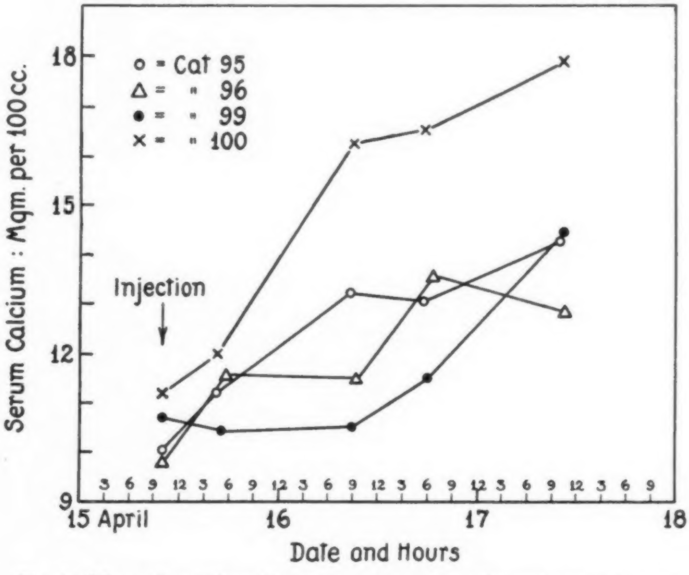

Fig. 1. Effect of parathyroid extract upon serum calcium in normal cats

ments; in cat 95, during the peak of the calcemia, the heart rate was seldom below 200 per minute. The animals with a moderate degree of hypercalcemia showed no signs of distress. In fact, they ate ravenously and consumed large quantities of water.

II. *Sympathectomized animals.* In table 2 are given figures for the basal serum calcium level in 6 sympathectomized cats and in 2 dogs, at periods from 1 month to 3 years after the operation. They are all within the normal range. Our findings agree with those of Lamelas (1930) to the effect that absence of sympathetic nerves has no apparent effect upon the calcium level in blood serum. Four cats and two dogs were given Parathormone

TABLE 1

Serum calcium and action of parathormone in normal animals

NUMBER	WEIGHT	NORMAL CALCIUM IN 100 CC. SERUM	TIME OF INJECTION	AMOUNT INJECTED	BLOOD SAMPLE TAKEN	CALCIUM IN 100 CC. SERUM	REMARKS
	kgm.	*mgm.*		*units*		*mgm.*	
Cat 96	2.2	9.8	Apr. 15, 9:40 a.m.	11			
			10:00 p.m.	10	Apr. 15, 5:40 p.m.	11.6	
			Apr. 16, 9:45 a.m.	10	Apr. 16, 9:30 a.m.	11.5	
			3:15 p.m.	15	6:20 p.m.	13.6	
			Apr. 17, 1:00 a.m.	15	Apr. 17, 10:45 a.m.	12.8	
Cat 100	3.5	11.2	Apr. 15, 9:40 a.m.	30			
			10:00 p.m.	10	Apr. 15, 4:45 p.m.	12.0	
			Apr. 16, 9:30 a.m.	10	Apr. 16, 9:00 a.m.	16.3	
			3:15 p.m.	10	5:50 p.m.	16.6	
			Apr. 17, 1:00 a.m.	15	Apr. 17, 10:15 a.m.	17.9	
Cat 99	4.6	10.7	Apr. 15, 9:35 a.m.	23			
			10:05 p.m.	15	Apr. 15, 5:20 p.m.	10.4	
			Apr. 16, 9:40 a.m.	15	Apr. 16, 9:00 a.m.	10.5	
			3:15 p.m.	20	6:00 p.m.	11.5	
			Apr. 17, 1:00 a.m.	20	Apr. 17, 10:30 a.m.	14.4	
Cat 95	3.0	10.1	Apr. 15, 9:35 a.m.	20			
			10:05 p.m.	10	Apr. 15, 4:30 p.m.	11.2	
			Apr. 16, 9:30 a.m.	10	Apr. 16, 8:45 a.m.	13.2	
			3:20 p.m.	10	5:30 p.m.	13.1	
			Apr. 17, 1:00 a.m.	15	Apr. 17, 10:00 a.m.	14.3	
Cat 93	2.5		Apr. 17, 11:00 a.m.	30			
			4:15 p.m.	30			
			11:15 p.m.	15	Apr. 18, 9:45 a.m.	15.8	

Cat 94	3.5		Apr. 17, 11:00 a.m.	40			
			4:30 p.m.	40			
			11:20 p.m.	15	Apr. 18, 10:25 a.m.	11.8	
Cat 100	3.6	11.2	Apr. 27, 1:00 p.m.	40			
			8:00 p.m.	20			
			Apr. 28, 11:30 a.m.	20			
			10:00 p.m.	30			
			Apr. 29, 2:00 p.m.	20	Apr. 29, 10:15 a.m.	17.4	No symptoms
					Apr. 30, 10:30 a.m.	15.6	No symptoms
					May 1, 10:45 a.m.	11.6	
Cat 95	3.0	10.1	Apr. 27, 1:00 p.m.	35			
			8:00 p.m.	20			
			Apr. 28, 11:30 a.m.	20			
			10:00 p.m.	30			
			Apr. 29, 2:00 p.m.	20	Apr. 29, 10:45 a.m.	17.2	No symptoms
			6:45 p.m.	50	Apr. 30, 10:15 a.m.	19.0	Marked muscular weakness; incoördination. Heart rate, 230, polypnea
					May 1, 10:00 a.m.	12.8	Still very weak
Dog N	12	10.4	Apr. 18, 5:00 p.m.	65			
			9:00 p.m.	35			
			Apr. 19, 7:45 p.m.	90	Apr. 19, 10:00 a.m.	13.0	
					Apr. 20, 12:00 a.m.	16.2	No symptoms

TABLE 2

Serum calcium and action of parathormone in sympathectomized animals

NUMBER	WEIGHT	SYMPATHECTOMY COMPLETED	NORMAL CALCIUM IN 100 CC. SERUM	TIME OF INJECTION	AMOUNT INJECTED	BLOOD SAMPLE TAKEN	CALCIUM IN 100 CC. SERUM	REMARKS
	kgm.		*mgm.*		*units*		*mgm.*	
Cat 294		Mar. 3, 1930	9.8					
Cat 295		Mar. 1, 1930	9.8					
Cat 107	3.7	Oct. 10, 1927	10.0	Apr. 17, 11:00 a.m.	30			
				4:30 p.m.	30			
				11:15 p.m.	10	Apr. 18, 10:15 a.m.	13.6	
Cat 384	3.5	Oct. 10, 1928	10.6	Apr. 17, 11:00 a.m.	35			
				4:15 p.m.	30			
				11:15 p.m.	10	Apr. 18, 10:00 a.m.	12.3	
Cat 456	2.3	Mar. 18, 1930	9.8	Apr. 27, 1:00 p.m.	30			
				8:00 p.m.	20			
				Apr. 28, 11:30 a.m.	20			
				10:00 p.m.	30			
				Apr. 29, 2:00 p.m.	20	Apr. 29, 10:40 a.m.	14.2	No symptoms
						Apr. 30, 10:35 a.m.	18.0	No symptoms
						May 1, 11:00 a.m.	14.2	No muscular weakness, slight incoordination
Cat 286	2.3	Feb. 18, 1930	10.2	Apr. 27, 1:00 p.m.	30			
				8:00 p.m.	20			
				Apr. 28, 11:30 a.m.	20			
				10:00 p.m.	30			
				Apr. 29, 2:00 p.m.	20	Apr. 29, 11:00 a.m.	13.4	No symptoms Apr. 30: found dead.
				6:45 p.m.	45			
Dog S	9.5	Jan. 1929	11.1	Apr. 18, 4:20 p.m.	50			
				9:15 p.m.	25			
				Apr. 19, 10:00 a.m.	40	Apr. 19, 10:00 a.m.	13.0	
				7:45 p.m.	90			
						Apr. 20, 12:00 a.m.	17.0	No symptoms
Dog B	15.8	Jan. 1929	10.6	Apr. 18, 4:30 p.m.	75			
				9:00 p.m.	40			
				Apr. 19, 10:00 a.m.	55	Apr. 19, 10:00 a.m.	13.7	
				7:45 p.m.	100			
						Apr. 20, 11:45 a.m.	16.7	No symptoms

in amounts stated in table 2. The effects of both small and large doses were quite similar to those noted in the normal cats, and in one normal dog used as a control. Two animals that received large doses showed toxic symptoms. In cat 456 these symptoms supervened after the blood calcium had reached 18 mgm. per 100 cc.; this animal recovered. Cat 286, given about 70 units per kilogram, died in the course of 3 days. It will be seen that, as compared with normal animals, sympathectomized cats show no marked change in susceptibility to parathyroid extract. The two operated dogs likewise showed no difference in reaction from the control. In one, about 8 units per kilogram increased the calcium from 11.1 mgm. per 100 cc. to 13.0 mgm. in 15 hours. A calcemia of 17 mgm. was induced by the further injection of 13 units per kilogram. Apparently, the sympathetic nervous system is not essential for the development of endogenous hypercalcemia. Palmer (1920) reported that double splanchnectomy in dogs did not prevent the typical response to parathyroid extirpation, including hypocalcemia and tetany. These conclusions, together with the facts that sympathectomized animals have a normal blood calcium content, and show no sign of parathyroid dysfunction, indicate that the sympathetic nervous system plays no essential part in what is known concerning the function of the parathyroid glands.

CONCLUSIONS

1. Normal cats show responses (increase of blood calcium, muscular weakness, polypnea, etc.) to parathyroid extract (Parathormone) essentially similar to that observed in dogs by previous investigators. Toxic symptoms occur after prolonged administration, but the time of onset does not correspond to the highest blood calcium level.

2. In sympathectomized cats and dogs the basal level of blood calcium is not outside the average range for normal animals (confirming the findings of Lamelas, 1930).

3. The absence of central sympathetic nervous impulses does not appreciably influence the response of cats and dogs to parathyroid extract.

We wish to express our thanks to Prof. W. B. Cannon for suggesting this problem.

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EXPERIMENTAL ARTERIOSCLEROSIS IN THE RAT

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The term arteriosclerosis will be used rather than any other because it is the most familiar and expresses adequately enough the malformations of the arteries studied. Arteriosclerosis in its wide sense signifies all those changes of the arteries which lead to a thickening of the wall, especially of the intima. In the process sclerosis and calcification may occur.

Hess (2) has shown that viosterol (irradiated ergosterol) is able not only to raise the level of the inorganic phosphorus and calcium of the blood when it is decreased to tetany level, but that it brings about hypercalcemia when given to normal infants or animals. Hess (3) later found by feeding large amounts of viosterol to rats low in blood calcium, that without exception the blood calcium could be rapidly increased 50 per cent or more.

Hückel and Wenzel (5) found that large daily doses of viosterol administered orally to rabbits caused the anterior portion of the aorta to become thickened and the inner surface to become roughened and ridged.

Hess (4) and fellow workers have recently completed a series of experiments in which infants were treated with varying doses of viosterol. Some of their doses were as high as seventy-five drops daily or seven and one half times the prophylactic dose recommended. They report no toxic effects found in even the highest dosage and concentration combinations, over limited lengths of time.

Bills and Wirick (1) recently published investigations on 1200 rats covering a period from infancy to maturity. Their results indicate in long time experiments that 100 times the prophylactic dose of viosterol is harmless, 1000 times over dosage is just perceptibly harmful, while 4000 times over dosage is definitely injurious.

Experimental. Normal healthy adult rats were used throughout. All animals were given clean water and the basal diet ad libitum. The basal diet was composed of the following: Yellow corn meal 65 per cent, meat and bone meal 10 per cent, hulled ground oats 10 per cent, flour middlings 8 per cent, powdered buttermilk 5 per cent, NaCl 0.5 per cent, fresh cod liver oil (added separately) 1.5 per cent.

The animals were weighed at regular intervals as a means of aiding in the estimation of general health.

Careful post-mortem examination, gross and microscopic, following spontaneous death or death by illuminating gas was the method of determining the presence or absence of arteriosclerosis. Microscopic slides were prepared by fixing the tissues in Zenker's fluid, sectioning 10μ in thickness and staining with eosinhaematoxylin. Double staining was used for the purpose of differentiating calcium deposits.

High salt diet group. Five adult, male, albino rats were fed a diet consisting of 85 per cent basal ration plus 5 per cent sodium chloride and 10 per cent sodium bicarbonate by weight. Two animals of like weight and age were kept as controls.

These animals consumed many times the ordinary amounts of water due to the high consumption of salts. At first they ate little, which occasioned loss of about one-sixth the initial body weight. This was slowly regained, until at termination of the experiment the weights were approximately that at the beginning. They developed a semi-diarrhea which caused them to appear gaunt and drained throughout the experiment. They were kept on the high salt diet for approximately seven months, after which time all animals were killed and careful autopsy performed.

Macroscopic examination revealed no malformations whatever either of the arteries or of the internal organs.

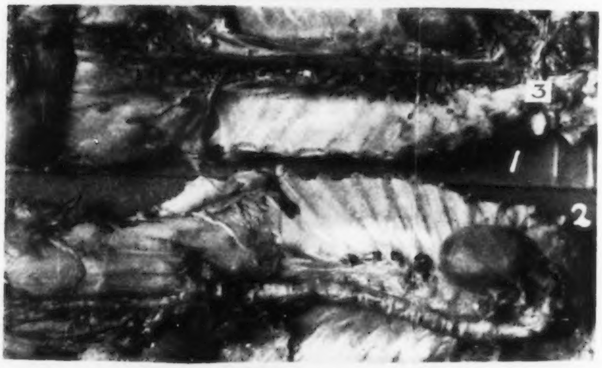
Histological sections were made of portions of the thoracic aortae. Upon microscopic examination no calcium could be discovered in any of the slides prepared.

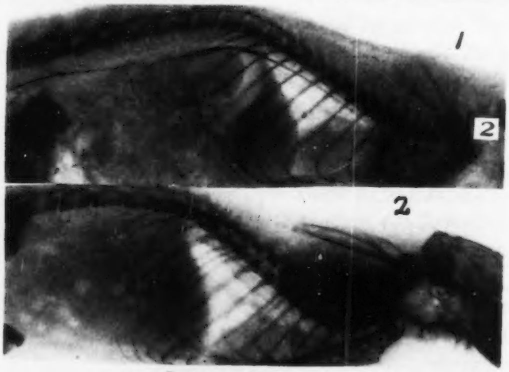
Staphylococcus aureus. A group of eight adult male rats averaging approximately 150 grams each were used. Bacterial suspensions for injection were prepared as follows: four agar slants were inoculated by spreading one streak from a stock culture of staphylococcus aureus on the surface by means of a sterile platinum loop. After twenty-four hours at 37°C. 2 cc. of Locke's solution were added to each slant, and the growth was loosened becoming suspended in the solution. One cubic centimeter of this suspension was injected into each rat, weekly, subcutaneously in the back of the neck.

A swelling followed each injection. This usually radiated, causing enlargement of the front legs. It never totally subsided before the next injection. Thus a constant state of infection was induced.

These animals were sluggish and slow in their action about the cages. They ate normally, but during the first month lost slightly in weight. This was later regained and by the termination of the experiment they excelled somewhat their initial weights.

The treatment was continued for seven months, none dying as a result. The animals were then killed and carefully examined. Macroscopically no pathology in the arteries could be seen, but, in all cases, the lungs showed an enormous amount of infection with quantities of pus present.





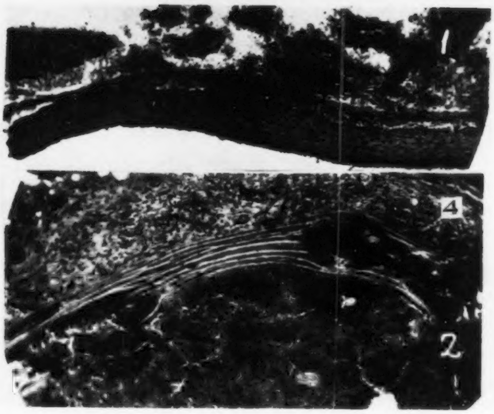


Fig. 1. First viosterol group after 58 days on experiment. *1*. Control; *2* and *3*, viosterol fed.

Fig. 2. X-ray. *1*. Aorta injected with $BaSO_4$ to check location in 2. *2*. Calcified aorta.

Fig. 3. Aortae in situ. *1*. Control. *2*. Sclerosed.

Fig. 4. Microphotographs of aortae cross-sections. *1*. Normal. *2*. Sclerosed.

Histological sections were made from portions of the thoracic aortae, but no evidence of sclerosis could be found.

Viosterol group. Nine adult male albino rats were placed on experiment in groups of three at different times. These animals weighed between 250 and 350 grams each.

The first group was placed on experiment in January 1930. They were fed the basal diet except cod liver oil was omitted and viosterol substituted. The viosterol was fed in a mixture of either cheese or bread crumbs as a vehicle. The first group received amounts varying between 1.5 cc. and 2.0 cc. daily.

The hair of these animals became very rough and coarse within the first ten days. It was gradually shed until the skin could be seen through it (fig. 1). They became extremely restless and irritable. Their appetites decreased and weight loss was so extreme that at the end of fifty days it was reduced to approximately 50 per cent of the initial. One animal died after fifty-five days, one after sixty-seven days, and the third was killed after seventy-four days on experiment.

X-ray pictures of the third showed a definite shadow in the position of the aorta from its beginning throughout the thorax and abdomen (fig. 2).

Careful post-mortem examinations were made of all these animals. The findings confirmed the x-ray indications. Dense annular calcifications were evident even to the branches of the iliacs. The aortae resembled tracheae in external appearances due to the calcium rings (fig. 3). The amount of calcification was in direct proportion to the amount of viosterol which had been consumed by the animals.

Histological slides were prepared from portions of the aortae. Microscopic examination of the layers revealed the intima to be the chief site of deposition with involvement of the media in the most extensively and thickly calcified areas. Slides were also prepared of a check animal of approximately the same size, for comparison (fig. 4).

The second group of three was placed on experiment April 1, 1930. These were cared for exactly as the first group except that they were given 2.5 cc. of viosterol daily. They presented outward symptoms which were perfectly analogous to the first group. At the end of twenty-five days x-ray photographs were made to determine whether the aortae were calcified. A definite shadow was found in all three cases. This deposition was of comparatively rapid onset. One animal was killed and examined macroscopically to check the x-ray photographs. The findings confirmed those of the x-ray.

The remaining two animals soon regained their initial weight and increased quite normally beyond it. Normal vigor and health were apparently regained. They were allowed to thrive unmolested for thirty days.

After the thirty days of no treatment the second animal of the group was killed. The object here was to determine whether or not a limited time factor alone would allow decalcification. Macroscopic examination of the animal revealed as highly sclerosed arterial system as had been presented by the preceding animals.

One animal with sclerosed arteries had thus been obtained which time alone would not decalcify. A means of decalcification was next considered. The administration of large doses of parathyroid hormone seemed logical in light of clinical evidence that hyperparathyroidism causes decalcification of the bones. The increased amount of calcium in the blood after the administration of the hormone might conceivably be derived from the abnormal deposits.

Collip's parathyroid (Lilly) hormone was injected into the third animal in 1 cc. doses, at varying intervals. The injections were made in the back of the neck, 15 cc. being given during a period of two months. Decreased vigor and activity were apparent in this rat. He again showed symptoms of irritability and lost slightly in body weight. Death was finally caused by administering three doses of parathyroid extract on three successive days.

At autopsy the animal exhibited a marked sclerosis of the entire aorta, the innominate and subclavians. This animal showed also marked malformation of the kidneys. It was the first case of an outstanding abnormal renal appearance noted. Instead of being the usual deep red color, the kidneys were strikingly mottled with fine grayish white blotches. This mottling was not only on the surface but extended through the cortex. Incision by a sharp scalpel gave evidence of calcified renal blood vessels.

The last group of this experiment was placed on experiment June 1, 1930. These animals were treated exactly as group two and their reactions were distinctly analogous. However, one animal died prematurely after six days and, as expected, showed no arteriosclerosis.

The remaining two animals were killed after receiving 2.5 cc. daily doses of viosterol for twenty-five days. At autopsy both exhibited cases of arteriosclerosis very similar to the preceding group.

Of the nine animals in the viosterol group, therefore, one died during the first week, and, as expected, showed no arteriosclerosis, the time being too brief; of the remaining eight, positive arteriosclerosis was present in all cases. The minimum experimental time before examination was made was twenty-five days. The minimum total amount of viosterol consumed was approximately 50 cc. There is no doubt but that the process of sclerosis was gradual and could have been seen, to some extent, with a less amount of viosterol and in a shorter time.

CONCLUSIONS

1. A high salt diet consisting of 85 per cent basal diet plus 5 per cent sodium chloride and 10 per cent sodium bicarbonate does not cause arteriosclerosis when fed to adult rats constantly for seven months.

2. A state of constant intensive infection for a period of seven months, induced by weekly injections of staphylococcus aureus, does not cause arteriosclerosis in adult rats.

3. The consumption of approximately 50 cc. of viosterol over a period of twenty-five days causes definite arteriosclerosis in adult rats.

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THE ACIDITY OF THE GASTRIC CONTENTS OF NORMAL, CRETIN AND HYPERTHYROID RABBITS[1]

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Our knowledge concerning the effect of experimental hypo- and hyperthyroidism on the acidity of the gastric juice has been obtained almost entirely from analyzing Pavlov pouch juice of dogs, either after extirpation of the thyro-parathyroid glands, or after the ingestion of thyroid substances. Reports on decrease in gastric acidity due to experimental hyperthyroidism are consistent, and the indications are that the animals were in a state of hyperthyroidism, despite the fact that no basal metabolism determinations were made. Relative to studies on experimental hypothyroidism the situation is different. First, only a few analyses of gastric acidity have been made; second, contradictory results are reported; third, there is nothing to indicate that the animals were actually in a state of thyroid deficiency, and lastly, parathyroid deficiency probably complicated the results, thus making the evidence at hand concerning the acidity of the gastric juice in uncomplicated hypothyroidism of such an unsatisfactory character, that the validity of whatever conclusions were drawn is open to question. This, together with the well-established fact that in the rabbit, total extirpation of the thyroid glands is not followed by parathyroid insufficiency, as determined by the level of the serum calcium (Kunde and Carlson, 1927) led us to attempt a study of the gastric acidity in cretin rabbits. Moreover, in cretin rabbits there develops an apparent cachexia and a characteristic anemia (Kunde, 1926) which in many respects simulates pernicious anemia. In man, such extra-gastric disturbances are accompanied by hypo- and anacidity. We were interested in finding out whether the same obtains in these cretin and anemic rabbits.

Method and experimental procedure: The cretins were produced by total extirpation of the thyroid glands approximately three weeks after birth. This technique has been previously cited (Kunde and Carlson, 1927). The gastric contents of these rabbits was not studied until four to six months after the operation, at which time marked signs of advanced cretinism had developed (Kunde and Carlson, 1927, Kunde and Neville,

[1] The present investigation was aided, in part, by a grant to the University of Chicago from the Rockefeller Foundation.

[2] Seymour Coman Fellow in Physiology.

1930. Hyperthyroidism was produced by feeding 260 to 290 milligrams of desiccated thyroid daily to each rabbit. In the hyperthyroid group, analysis of the gastric contents was made after severe hyperthyroidism was apparent (two to six weeks after daily thyroid ingestion).[3]

Since previous attempts at converting a portion of the rabbit's stomach into a Pavlov pouch have proved futile, we were obliged to limit our studies to analysis of the acidity of the aspirated gastric contents. Our analysis, therefore, in no instance gives absolute values for either the maximal or minimal acidity of the pure gastric juice in rabbits. Every specimen was

TABLE 1

NaOH method

CONDITION OF ANIMAL	NUMBER OF ANIMALS	NUMBER OF DETERMINATIONS	PER CENT ACID IN GASTRIC CONTENTS						COMMENTS
			Highest		Lowest		Average		
			Free	Total	Free	Total	Free	Total	
Creatin	7	69	0.41	0.57	0	0.16	0.13	0.35	Without histamine
	4	13	0.42	0.56	0.17	0.26	0.27	0.41	30′ to 60′ after ½ mgm. histamine subcutaneously
Normal	6	29	0.42	0.55	0	0.21	0.13	0.32	Without histamine
	6	11	0.34	0.52	0.09	0.28	0.26	0.42	30′ to 60′ after ½ mgm. histamine subcutaneously
Hyperthyroid	5	21	0.16	0.49	0	0.17	0.04	0.29	Without histamine
	5	13	0.26	0.43	0	0.21	0.12	0.33	30′ to 60′ after ½ mgm. histamine subcutaneously

slightly contaminated by minute particles of food material and probably diluted by either saliva or regurgitated duodenal contents, or both. We hope that at some future date, a technique will be developed whereby pure pouch juice may be obtained from rabbits, the same as in dogs.

In our preliminary experiments, we attempted to diminish the food contamination factor by starving the rabbit for several days. This, under the ordinary dietary régime of the rabbit (alfalfa hay, carrots, oats, and water *ad libitum*) proved futile. Post-mortem examination of rabbits four to six days after total abstinence from food revealed large quantities of food material in the stomach. We therefore decided to aspirate the

[3] Desiccated thyroid, Armour and Company, U. S. P.

stomach in the morning just before the daily feeding, either under normal conditions, or 30 to 60 minutes after injecting 0.5 mgm. of histamine hydrochloride subcutaneously.

The acidity of the aspirated gastric contents was determined by direct titration with standard NaOH, using dimethyl-amino-azo-benzol (Topfer's reagent) for the free acid, and phenolphthalein for the total acid. The amount of HCl present (free and combined) was ascertained by the Hayem and Winter method for determination of HCl of gastric juice.

RESULTS AND DISCUSSION: Table 1 contains a summary of our results on the acidity of the gastric contents as determined by direct titration with

TABLE 2

Hayem and Winter method

Condition of animal	Number of animals	Number of determinations	Per cent HCl in gastric contents						Comments
			Highest		Lowest		Average		
			Free	Total	Free	Total	Free	Total	
Creatin	5	23	0.25	0.55	0	0.12	0.06	0.29	Without histamine
	4	9	0.27	0.51	0.02	0.24	0.14	0.38	30′ to 60′ after ½ mgm. histamine subcutaneously
Normal	6	17	0.21	0.55	0	0.13	0.09	0.28	Without histamine
	6	8	0.31	0.53	0.08	0.18	0.19	0.36	30′ to 60′ after ½ mgm. histamine subcutaneously
Hyperthyroid	3	14	0.09	0.33	0	0.14	0.03	0.25	Without histamine
	7	13	0.11	0.41	0	0.12	0.05	0.32	30′ to 60′ after ½ mgm. histamine subcutaneously

standard NaOH. Table 2 shows the actual amount of HCl present as free and combined acid. The close agreement between the results for total acidity found by these two methods, indicates that the acid present in the gastric contents of the cretin, hyperthyroid and normal rabbits is due largely to HCl, only insignificant amounts of other acids being present. Furthermore, eleven quantitative determinations for lactic acid were made[4] which ranged from 23 to 37 mgm. per cent of the gastric contents, the cretins showing no more than the normal or hyperthyroid rabbits. The occasional discrepancies found between the direct titration with NaOH and the Hayem and Winter method may be explained by the fact that, in

[4] Analysis made by Dr. T. E. Friedeman, by the Friedeman, Cotonio and Shaffer method.

drying, a small amount of combined HCl may be liberated, and in the ashing, acid phosphates may be formed, which expel some of the chlorine from the NaCl.

These analyses clearly indicate that the concentration of HCl in the gastric contents of cretin rabbits, with anemia and cachexia, is neither depressed below that of normal rabbits, nor is it increased. Chang (1927) reports on the gastric secretion of two Pavlov pouch dogs after thyroidectomy, and notes a demonstrable increase in the acidity of the pouch juice following the operation, although the tabulated results indicate that the post-operative gastric acidity of these dogs is well within the range of acidity found in most normal dogs. Dragstedt (1927) studied the Pavlov pouch juice of thyroparathyroidectomized dogs and found that the secretion of gastric juice is unaffected when the tetany is controlled. Boenheim (1920) reports clinical cases after thyroidectomy, and notes a marked achylia in some cases of post-operative myxedema, but since the acidity of the gastric contents of his patients before the disturbance of the thyroid gland had not been determined, it is possible that complications other than thyroid deficiency may have contributed to his findings.

The slight but appreciable depression in the HCl of the gastric contents of rabbits, with experimentally induced hyperthyroidism (tables 1 and 2) conforms to the decreased gastric acidity of Pavlov pouch dogs with hyperthyroidism reported by Hardt (1916), Truesdell (1926), and Chang (1927).

SUMMARY

The concentration of HCl of the gastric contents (normal and histamine induced) of cretin rabbits, with severe anemia and cachexia, is the same as in normal rabbits.

The HCl of the gastric contents of rabbits with experimental hyperthyroidism is depressed but not absent.

The total HCl of the gastric contents of normal and cretin rabbits ranges from 0.2 to 0.56 per cent.

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STUDIES ON METABOLISM

VIII. The Effect of Estrin Injections on the Basal Metabolism, Uterine Endometrium, Lactation, Mating and Maternal Instincts in the Adult Dog[1]

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Several adequate reviews on the influence of the ovaries and ovarian substances on the basal metabolic rate are available in the literature. For a detailed account of these the reader is referred to the splendid compilation of abstracts by Zunz (1924), the book by DuBois (1927), and the recent article by Hitchcock (1930). These reports include observations made on women at puberty, during menstruation, at the menopause, after surgical removal of the ovaries and following the administration of ovarian preparations. References to the above will not be made again in the limited space of this report, unless some of our findings seem to have direct bearing on the results reported for women.

The monograph by Frank (1929) contains a resumé of the numerous articles dealing with changes in the female reproductive system, or closely associated physiological processes, following the administration of extracts containing the female sex hormone (estrin). Some of these are closely correlated, if not identical, to our observations on congestion of the external genitalia, changes in the uterine endometrium, hypertrophy of the mammary glands; psychic manifestations and pronounced evidence of the maternal and mating instincts in adult dogs, following the administration of estrin.

In females of lower species experiments on the effect of the ovaries or ovarian substance on the basal metabolism include observations made during estrus, Kunde (1923) Lee (1928), and after the removal of the ovaries, Lütje (1902), Bugbee (1926).

The investigations of this report were undertaken because no studies on the effect of estrin on the basal metabolism of dogs have been previously reported.

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[2] Seymour Coman Fellow in Physiology.

METHODS AND RESULTS. *Preparation and assay of female sex hormone.* The hormone used in these experiments was obtained from human pregnancy urine according to the following procedure:

Urine, made acid to Congo Red, was extracted with butyl alcohol in a continuous extractor for 48 hours. The alcoholic extract was taken to dryness, the residue dissolved in ether and the ether extracted 3 times with a saturated solution of sodium bicarbonate. The ether was removed by distillation and the residue dry-distilled under reduced pressure at 160°C. to remove butyl urethane. The residue was redissolved in ether, cooled to −12°C. and filtered through cotton. The ether solution was taken to dryness and the residue leached out thoroughly with a 5 per cent solution of sodium hydroxide. The alkaline solution was cooled to −12°C. and filtered, acidified till very faintly alkaline to phenolphthalein and extracted 6 times with ether. This solution was assayed and made up for injection by pouring into olive oil and removing the ether by evaporation on a steam bath.

Assay. The method used was the vaginal smear test of Allen and Doisy (1923). For the purpose of this experiment no smear was considered positive unless there was a complete replacement of leucocyte cells by squamous epithelial cells. The unit used is the one regularly employed in these laboratories on routine assays, namely, that amount of material which will bring 3 rats into complete estrus providing the next higher dosage used does the same for 3 other animals. This preparation assayed as follows: 0.1 mgm., E, E, E; 0.08 mgm. E, E, E; 0.06 mgm. E, E, E; 0.04 mgm. E, E, E; 0.02 mgm. E, EL, LE.[3]

Repeat: 0.06 mgm. E, E, E; 0.04 mgm. E, E, E; 0.02 mgm. E, E, E.

Therefore we consider 0.04 mgm. of this material as equal to one rat unit (D'Amour and Gustavson, 1930).

Experimental procedure and results of estrin injections in adult female dogs. The estrin prepared and assayed as above was injected subcutaneously into 4 bitches weighing 10 to 12.6 kilos. Two of these were normal, and 2 were completely castrated bitches. The daily dose that each dog received was either 100 or 200 rat units. The periods of injection varied from 10 to 39 days. The injections were followed by no signs of untoward effects at the site of injection despite the fact that as many as 39 subcutaneous injections were made in an area approximately $2\frac{1}{2}$ by $1\frac{1}{2}$ inches.

Basal metabolism determinations were made daily on 2 of the normal dogs during the period of estrin injections. One of these received 100 rat units daily for a period of 39 days and the other 200 rat units daily for an equal duration of time. The dogs were chosen from the well-trained animals of the metabolism series, which had been housed in the laboratory for

[3] "E" signifies a positive reaction, "EL" a mixed smear with epithelial cells predominating, "LE" a mixed smear with leucocytes predominating.

many months and on which many metabolism tests under normal conditions had been made (Kunde, 1927). The method employed in determining the basal metabolism has been previously described (Kunde, 1922), (1923); (Kunde and Steinhaus, 1926), excepting this detail, which is an easily applied and additional safeguard against leakage at the point of contact between the dog's nose and the rubber diaphragm of the muzzle. A piece of rubber dam (the same grade as is used in the muzzle), approximately 1 × 3 inches, is tightly pulled over the broad rim of contact between the dog's nose and the muzzle. The two ends are tightly held together by clipping a broad hemostat smoothly over their entire width.

The variations in basal metabolism, body temperature, body weight and pulse rate resulting from the subcutaneous injection of the female sex hormone, for 39 consecutive days, are well within the limits of variations which occur under normal conditions (±5 per cent for the basal metabolism). An exception in body temperature occurred in dog IE over a period of approximately 5 days. This will be fully discussed under lactation and maternal instinct. No basal metabolism tests were made on the first 3 of these 5 days (15th, 16th, and 17th days of estrin injections). Several days of slight elevation in body temperature (0.5°–0.6°F.) followed this with corresponding metabolism tests near the upper limit of normal variation. These tests slightly increase the average (+2 per cent) over the time of estrin injections. Other than this, the basal metabolism for the entire period is characterized by low normal tests. This same fact holds for the results observed in dog IIE, receiving 200 rat units of hormone daily. The data indicate that a predominating number of low normal tests, result in an average of −2 for the entire experimental period, but with no test below the lower limits of normal. These low normal results are strikingly comparable to the low normal tests which occur in the dog during spontaneous estrus (Kunde, 1923) and gives evidence of the fact that experimental estrus, in the dog, induced by subcutaneous injections of the female sex hormone has the same effect on the basal metabolic rate as the normal physiological process.

A discussion of the similarities and dissimilarities of the factors involving menstruation in primates and estrus in dogs, is entirely outside the scope of this report. But we mention here that increasing data indicate that in many normal women the onset of the menstrual flow is followed by several days of basal metabolism tests near the lower limit of normal variation (Kunde, 1923; Hitchcock, 1930).

Mating instinct; congestion and hemorrhagic discharge from the external genitalia. All observations described in the following paragraphs were made on 4 dogs. Of these, 2 were normal animals (ovaries intact) already mentioned (dogs IE and IIE, receiving daily doses of 100 and 200 rat units of hormone respectively for 39 consecutive days). The other 2 animals,

dogs M and S, were complete castrates. Bilateral ovariectomy was performed on dog M, January 31, 1930. On February 18, 1930, the greatest extremities of the labia measured 16 × 21 mm.; 100 rat units of female sex hormone were injected daily on February 18th, 19th and 20th. No apparent effect was observed as a result of this. Beginning February 21st, the daily dose was increased to 200 rat units. On February 22nd the labia presented a gaping appearance and a measurable increase in length could be demonstrated. A muco-sanguineous discharge from the vagina was first observed February 23rd, and in the presence of normal male dogs, mating behavior became pronounced. On February 24th acceptance of the male was observed. The greatest extremities of the labia measured 24 × 31 mm. on this date and remained approximately at this size throughout the experiment. (Hormone discontinued February 28th.) The mating behavior, congested appearance of the external genitalia, and sexual interest, manifested by the normal male, persisted throughout the period of hormone administration, and for several days after its discontinuance. On March 10, 1930, the extreme dimensions of the labia were again 21 × 16 mm. No evidence of the mating instinct could be detected and no sexual interest displayed by the normal male. A second series of hormone injections was instituted on this day, extending over a period of time 33 days in duration (March 10, 1930, to April 12, 1930). The hormone administered over this second period of experimentally induced estrus was 100 rat units daily. The other ovariectomized female, dog S, received daily injections of 100 rat units of hormone for 28 days. The two normal females, dogs IE and IIE, received dosages already mentioned over a period of time 39 days in duration.

A striking uniformity of results characterize the effect of estrin injection in these 4 adult dogs, including the second period of hormone administration in dog M, the only significant variation being a difference of 3 or 4 days in the time of onset of comparable phenomena, due to difference in size of the dosage and biological response of the several animals (see table 1 for detailed data of dog IE and the chart for data of dog IIE). The swollen genitalia and intense sexual instinct, once evoked, persisted with no evidence of resolving (copulation was observed in dog IIE on the 39th day of experimentally induced estrus) until after discontinuing the hormone. The time required for the manifestations of rut to completely disappear after interrupting the estrin injections seems to be a factor of the size of the dose and the period of time over which the injections were made. Behavior characteristics indicative of estrus were still in evidence in dog IIE on the 14th day after discontinuing the hormone. Assays made on daily 24 hour urinary collections of this dog, previous to administering the hormone (February 28, 1930, to March 5, 1930) show no rat units per day. On the first day after injecting hormone (200 rat units) assay of the total 24 hour

TABLE 1

From the protocols of dog IE showing control data for 10 days (February 24, 1930, to March 5, 1930) preceding the administration of female sex hormone, and data during subcutaneous injection of 100 rat units of estrin daily for 39 consecutive days (March 5, 1930, to April 12, 1930)

DATE (1930)	BODY TEMPERATURE IN	PULSE PER MINUTE	WEIGHT	TOTAL CALORIES PER 24 HOURS	REMARKS
Control data					
	°F		kgm.		
February 24	100.8	68	12.1	470	Animal under normal conditions
February 25	101.0	64	12.3	480	
February 26	100.8	56		468	
February 27	101.0	72		480	
February 28	101.4	74	12.2	492	
March 1	101.0	88	12.2	462	
March 2	100.8	76	12.2	447	
March 3	100.8	84		479	
March 4	100.4	86	12.3	498	
Inject 100 rat units of estrin daily					
March 5	100.4	64	12.3	487	Labia measured 31 × 30 mm.
March 6	100.4	96	12.3	477	
March 7	99.8	70		470	
March 8	99.9	68		449	
March 9	100.6	72	12.3	489	Mucopurulent-looking discharge from vagina
March 10	100.0	60		465	Posterior pair of mammary glands secreting
March 11	99.8	78	12.1	467	Labia 45 × 35 mm.
March 12	100.8	78	12.1	478	Colostrum expressed from 2 more nipples
March 13	100.4	80	12.1	459	
March 14	100.5	72	12.2	488	Hemorrhagic discharge from vagina. Sex behavior
March 15	100.4	74	12.3	471	All 10 mammary glands lactating
March 16	100.4	74		484	Hemorrhagic discharge continues
March 17	101.0	72		472	
March 18	101.0	78	12.3	482	
March 19	100.9	74	12.3	474	Hemorrhagic discharge. Copulation observed. Suckles 2 puppies
March 20	103.0	92			
March 21	102.8	98			
March 22	102.6	98			

TABLE 1—*Concluded*

DATE (1930)	BODY TEMPERATURE IN	PULSE PER MINUTE	WEIGHT	TOTAL CALORIES PER 24 HOURS	REMARKS
	°F		kgm.		
March 23	101.2	80	12.0	496	
March 24	101.6	88		518	
March 25	101.6	84		496	
March 26	101.2	88		498	
March 27	101.8	76			
March 28	100.8	80			
March 29	101.0	92		498	
March 30	101.0	88	12.0	495	Sex behavior and lactation continue
March 31	100.8	76		495	Puppies have doubled their weight
April 1	100.8	74		492	Vaginal discharge contains less blood
April 2	101.0	72	12.1	492	
April 3	101.2	78		484	
April 4	100.0	88		498	
April 5	101.4	66		490	
April 6	101.2	64		496	
April 7	101.0	82		490	
April 8	100.8	74	12.0	492	
April 9	100.8	74		489	
April 10	100.6	68		474	Mating and maternal instincts persist
April 11	100.8	68	11.9	476	Vaginal discharge is thin and serous with little or no blood. Estrin discontinued
April 12	100.6	72	11.9	471	

urinary output showed 20 rat units. For the following three days, according to the assay, variations between 30 and 50 rat units were excreted daily. From the 39th day of hormone administration until the 3rd day after its discontinuance, 80 to 125 rats units were recovered daily from the total urinary output. Steadily decreasing amounts were eliminated thereafter. On the 7th day after discontinuing the subcutaneous injection, 35 rat units were recovered, and on the 13th day only 10 rat units were eliminated in the 24 hour urinary output.

The vaginal discharge of these adult dogs showed characteristic changes at different phases of estrus. A discharge was first observed on the 2nd to 5th day of injection, as a viscous, creamy-looking material which was replaced during the next 2 to 4 days by a thin blood stained discharge, rapidly becoming more sanguineous, until it simulated the characteristic hemorrhagic discharge of spontaneous rut. This persisted into the third week.

After this, as the period progressed, the amount of blood discharged decreased, until in those dogs with time extending up to the 39th day, only a thin serous macroscopically bloodless discharge could be detected. These findings seem significant when correlated with the length of the period of spontaneous rut in dogs (about 3 weeks) and point to a mechanism controlling one phase (bleeding from the genitalia) in the complicated process of estrus, which ceases to function under the same prolonged excessive

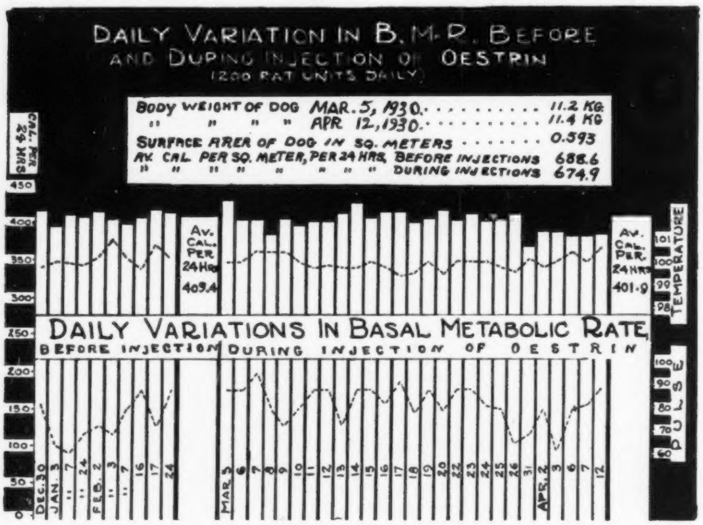

Fig. 1. Graphic presentation of the daily variations in basal metabolism, pulse rate, body temperature, and body weight of dog IIE, during a control period of 10 days immediately preceding the administration of female sex hormone and over a period of 39 consecutive days of subcutaneous injections of 200 rat units of estrin daily. The narrow bars indicate the total number of calories of heat produced per 24 hours for this dog. The broad bars indicate the averages for each period. The surface area is determined from the formula $^{3}\sqrt{W^{2}} \times 0.112$.

stimulus which initiated it, despite the fact that other phases of the same processes (swollen genitalia and intense mating instinct) do not resolve.

Laboratory records obtained from dog IE, previous to the observation of this report, denote a spontaneous estrus extending from December 12, 1929, to December 30, 1929, approximately nine weeks, before beginning the estrin injections. As this article goes to press (September 1, 1930) recurrence of a spontaneous estrus has not been in evidence. The estrus cycle in the normal dog recurs approximately every 6 months. Whether this delay is a result of the hormone injections, or an irregularity which

frequently obtains in the cycle of normal dogs cannot be determined at this time, but is mentioned here as a carefully observed fact, which may become more significant with increasing data on this type of research.

Uterine endometrium. Six weeks after surgical removal of both ovaries and six days before beginning the estrus injections, a piece of the middle third of the left uterine horn was removed from dog S, for histological

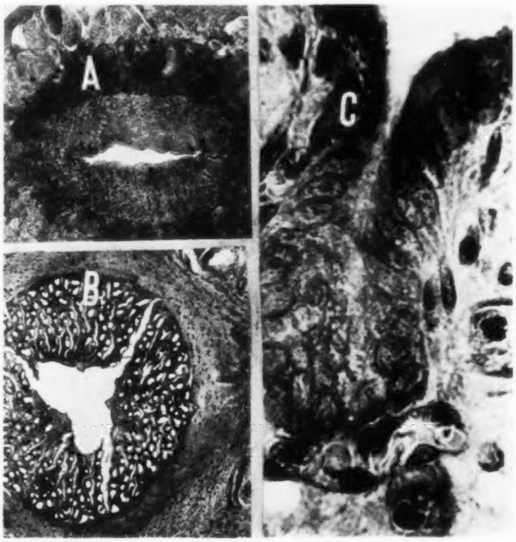

Fig. 2A and 2B. Showing photomicrographs (same magnification) of cross sections through the uterine horns of dog S; 2A, = castrate control condition 6 weeks after removal of both ovaries. 2B, = cross section through the contralateral uterine horn of the same castrate bitch, after subcutaneous injection of 100 rat units daily for 21 consecutive days. Note the hypertrophy of the glandular epithelium and the increase in thickness of the uterine muscle.

Fig. 2C. High power (× 1600) magnification of 2B, showing granules in glandular epithelium.

examination of the uterine endometrium in the castrate condition (see fig. 2, A). On the 21st day of injections, biopsy of the contra-lateral uterine horn in the same third was made. Figure 2, B, shows the marked hyperplasia of the glands of the endometrium, and the increased thickness of the uterine muscle at this time. Figure 2, C, is a high power magnification indicating secretory activity as evidenced by the granules in the glandular epithelium.

Lactation and maternal instincts. The effectiveness of the female sex hormone as a stimulus to the physiological processes involving lactation varied widely in these dogs. Hyperplasia of the nipples was a constant finding in all dogs. Doubtful to pronounced hypertrophy of the mammary glands, resulting in no secretion of milk to a copious flow as occurs after the normal process of gestation and delivery (see fig. 3) was observed. In dog IE, on the 5th day after beginning hormone injection, the posterior and largest pair of mammary glands began secreting. Two days later colostrum could be expressed from the two adjacent glands, and within the next three days milk was expressed from all 10 nipples. Six days later, two puppies

Fig. 4. Dog IE, mothering another dog's puppies

Fig. 3. Showing mammary glands of dog IE at height of lactation

(delivered by a different female 8 days previously) were mothered and suckled for three weeks by this dog, the puppies receiving no other nourishment during the three weeks but the milk which they obtained from her mammary glands. At the end of the three weeks the puppies weighed approximately three times as much as at the beginning. They had thrived apparently as normal puppies receiving their own mother's milk (see fig. 4).

The maternal instinct aroused in this dog immediately upon receiving the puppies is of unusual interest. She at once began cleaning and caring for them and arranging herself in a favorable nursing position. Her inter-

est and care for them was such that on the following morning her body temperature was 2°F. above normal. Relieving her from the care of the puppies for six hours resulted in a return to normal of her body temperature. A lesser degree in elevation of body temperature was observed on the following 5 days, which could invariably be reduced by separating her from the puppies for several hours. Except for the estrus periods this dog was very gentle and friendly in her attitude toward everyone about the laboratory. After receiving the puppies, fierce, askance looks were directed toward almost everyone entering the room and attempts at handling the puppies meant risking being bitten.

Manifestations of psychic disturbances, in dog IE, following the onset of spontaneous rut are strikingly comparable to the change in behavior of this dog after estrin injections. Her gentle and friendly attitude towards laboratory workers, under ordinary conditions, has already been mentioned. Her protocols show that during spontaneous rut special precautions for her isolation were enforced because of her attempts at biting even well-known laboratory workers. The same condition holds for the experimental estrus, even before the puppies were given her.

Our sincere thanks are due Prof. G. W. Bartelmez for assistance in the histological interpretation.

SUMMARY

The basal metabolic rate in normal dogs is neither lowered nor elevated as a result of subcutaneous injections of female sex hormone (estrin). Many tests near the lower limit of normal variation occur, so that the average over a period of daily injections may be slightly (2 per cent) below the average for a control period.

Congestion and enlargement of the external genitalia, hemorrhagic discharge from the vagina, hypertrophy of the uterine endometrium with histological evidence of secretory activity, and evocation of the mating instinct follow the administration of female sex hormone in the normal and castrate bitch.

Hyperplasia of the nipples and slight palpable enlargement of the mammary glands occur in all dogs, and copious lactation and evocation of the maternal instinct in one dog followed the subcutaneous injection of estrin.

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THE EFFECTS OF SIMULTANEOUS INJECTIONS OF THE FEMALE AND MALE HORMONES IN CAPONS[1]

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Since the chemical preparation of the two sex hormones has been accomplished, it is possible to study the effects of combinations of these hormones by administering known quantities and over known periods of time to the castrate.

The present account is concerned with the effects of the simultaneous presence of the chemically prepared female and male hormones in Brown Leghorn capons.

The sex dimorphism exhibited by this breed is such, that any deviation from the normal response of the dependent sex characters pertaining to either hormone may readily be noted. Such modifications, if occurring, would argue for an antagonism of the sex hormones.

The biological observations were carried out by one of us (M. J.) as part of the program on the biology of sex now being continued under the direction of Prof. F. R. Lillie at the Whitman Laboratory of Experimental Zoölogy, the University of Chicago.

The hormones were prepared in the Department of Physiological Chemistry, the University of Chicago. (The female hormone by F. D'A., the male hormone by E. B. W.)

The birds used were all caponised by Dr. L. V. Domm of Whitman Laboratory: we which to express our acknowledgments of his coöperation

The experiments were planned to check the following points: 1. Is there an "antagonism" in the organism of the chemically prepared hormones? 2. If not, may the effects of either be diminished or intensified by the presence of the other in the organism? 3. Do the hormones become modified on mixture "in vitro" for 24 and 48 hours?

Records of the kind desired require definite biological values for each hormone: these values then may be balanced against each other. Such standards of comparison for the two hormones have been established in

[1] The expenses of this investigation were supported in part by the Committee for Research in Problems of Sex of the National Research Council; grant administered by F. R. Lillie.

the Brown Leghorn. In the female hormone it is the amount, injected daily, which will induce female replacing plumage in the capon (Juhn and Gustavson, 1930). In the male hormone it is the amount, injected daily which will cause satisfactory comb growth in the capon (McGee, Juhn and Domm, 1928).

Experimental. The male hormone used was standardized in advance in bird units: it contained by this assay three bird units per cubic centimeter. ("A bird unit is defined as the amount of the hormone which, when injected daily for five days, yields an average of five millimeters increase in length and height of the combs of at least five Brown Leghorn capons." T. F. Gallagher and F. C. Koch. In press.)

The male hormone, W504, was also tested in a series of experiments on capons involving a study of the headfurnishings. In these preliminary tests, where the male hormone was administered alone, good comb growth was uniformly obtained of the same general order as in the experiments described here.

The female hormone was first tested for effectiveness in inducing female plumage in capons on a sample prepared from a stock solution and proved positive: this sample, no. 1356A, was also standardized in advance by the vaginal smear method, it contained by this assay 100 rat units per cubic centimeter.

A large amount of hormone was then prepared from the same stock in the same manner for use in these experiments and a number of others carried on about the same time in which plumage changes were studied. These other tests showed that the extract used, no. 1375, was not as effective in causing female feathering as the sample with which the preliminary readings were obtained. However, the amounts injected daily were adequate to call forth female plumage in definite regions.

Five capons were used in this series, of the same age and all castrated before puberty and kept in the laboratory under identical conditions.

Each capon was plucked in four different regions of the body: 1, in the breast, lateral at the level of the shoulder (anterior breast); 2, in the breast at the extreme lateral and posterior margin (posterior breast); 3, in the back at the level of the insertion of the wing, and 4, in the lateral and posterior margin of the saddle.

These four regions were selected because they have been found to show different threshold values in responding to the female hormone. The order of threshold values for these regions is: posterior breast > anterior breast > saddle ⇄ back.

The injections were given subcutaneously daily and continued for about one month. Each capon was weighed and the length and depth of its comb measured before beginning treatment, the same records were obtained at weekly intervals thereafter. All the birds were in good condition during the entire experimental period.

Experiment I. *Hormones mixed just before injection.* Capon 29–100 received daily a mixture of 1.5 cc. of the female hormone plus 1 cc. of the male hormone for 12 days and 2 cc. of the female hormone plus 1 cc. of the male hormone for the succeeding 22 days. The comb measurements and body weight of the capon during the time of injection and one month after the injections were discontinued are tabulated below.

DATE	COMB		WEIGHT	
	L.	D.		
	cm.	*cm.*	*grams*	
1–10–30	4.9	2.2	1798	Injections begun
1–17–30	6.0	3.0	1734	
1–24–30	6.5	3.3	1722	
1–31–30	7.1	3.8	1756	
2– 7–30	7.3	4.0	1779	
2–13–30	7.5	4.0	1770	Injections ceased
3–13–30	6.2	2.8	1865	

The headfurnishings showed good growth during the time of injections and both comb and wattles became red and turgid. See figure 1. One month after the injections were interrupted the comb size had regressed and the headfurnishings were pale and shrivelled.

The regenerating feathers were female in anterior breast, back and saddle. Two months after plucking and a month after the injections were discontinued the replacing plumage was almost fully developed and samples of the regenerating feathers were plucked for records. See figures 2, 3, 4, 5.

The simultaneous development of female plumage and growth of the headfurnishings demonstrate the independent action of the female and male hormone in the organism in these respects.

Experiment II. *Hormones injected separately.* Capon 29–31 received daily separate injections of female and male hormones. One and eight-tenths cubic centimeters of the female hormone was injected on the left side during the first 14 days; in the subsequent 19 days the daily dosage was increased to 2.5 cc.; 1 cc. of the male hormone was injected daily on the right side for the entire period.

The comb measurements and body weight of the capon for the time of injections and one month after treatment was interrupted are given below. The steady increase in comb size during the injections and regression after these were interrupted may be noted.

DATE	COMB		WEIGHT	
	L.	D.		
	cm.	*cm.*	*grams*	
1-10-30	5.3	2.7	2030	Injections begun
1-17-30	6.5	3.5	2027	
1-24-30	6.5	3.6	2015	
1-31-30	6.9	3.9	2018	
2- 7-30	7.1	4.0	2036	
2-13-30	7.3	3.9	2027	Injections ceased
3-13-30	6.2	3.0	1754	

The incoming feathers in anterior breast, back and saddle were female. One month after the injections were discontinued the regenerating plumage was well developed and feathers were then plucked and preserved for records.

The effects on feathering and headfurnishings here were quite similar to the results obtained in experiment I, the replacing plumage was female, the headfurnishings progressively assumed the male appearance during the period of injections.

Experiment III. *Hormones mixed for twenty-four hours previous to injection.* Capon 29-49 received daily injections of a mixture of the female and male hormones made 24 hours previously: 1.5 cc. of the female hormone plus 1 cc. of the male hormone for the first 12 days: in the succeeding 20 days the proportion of the female hormone was increased to 2 cc. plus 1 cc. of the male.

The records for comb growth and body weight during the period of injections and one month after these were discontinued are compiled below.

DATE	COMB		WEIGHT	
	L.	D.		
	cm.	*cm.*	*grams*	
1-10-30	4.0	1.9	1868	
1-11-30				Injections begun
1-17-30	4.2	2.1	1779	
1-24-30	4.6	2.4	1731	
1-31-30	4.8	2.5	1707	
2- 7-30	4.7	2.5	1663	
2-13-30	5.1	2.6	1611	Injections ceased
3-13-30	4.4	2.2	1731	

The incoming plumage was female in back and saddle. The regenerating breast feathers were male. In this capon the growth of the headfurnishings was not so pronounced as in the two preceding cases and female plumage was obtained only in the regions having a low threshold value for the

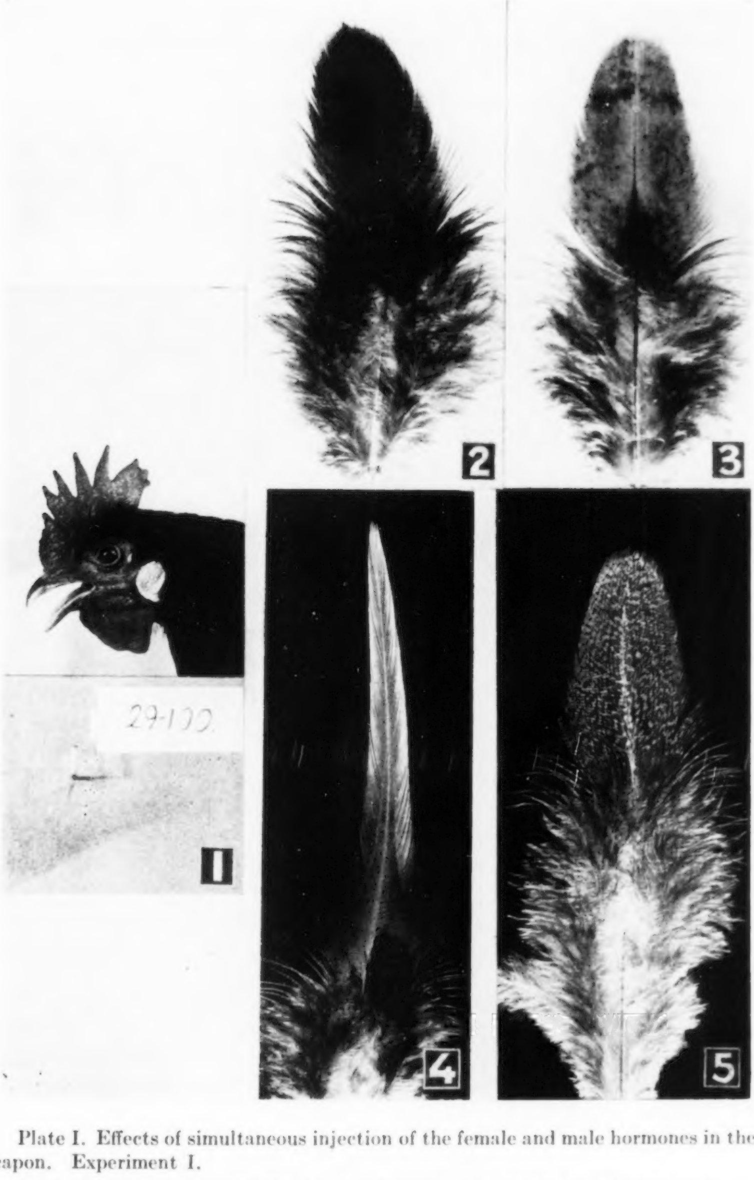

Plate I. Effects of simultaneous injection of the female and male hormones in the capon. Experiment I.

1. Photograph of capon 29-100 showing development of male type headfurnishings after one month of injection. Comb length, 7.5 cm., depth, 4.0 cm. Original comb dimensions, length, 4.9 cm., depth 2.2 cm.

2. Male breast feather from region plucked before beginning injections.

3. Female, replacing, breast feather from same region.

4. Male saddle feather from region plucked before beginning injections.

5. Female, replacing, saddle feather from same region.

female hormone. Plumage records were made one month after injections were discontinued.

These findings at first appear indicative of some attenuation of either hormone upon mixing "in vitro" for 24 hours. A comparison, however, with experiment IV, where the hormones were mixed for forty-eight hours previous to injection shows that there is no diminution of the intensity of the action of the hormones. The lesser response of capon 29–49 to either hormone must be attributed to individual variation of this bird: it may be noted that the capon lost considerable weight during the time of injection though the bird's general condition continued good.

Experiment IV. *Hormones mixed for forty-eight hours previous to injection.* Capon 29–71 received daily injections of a mixture of 1.5 cc. of the female hormone plus 1 cc. of the male hormone prepared 48 hours previously for 11 days, in the succeeding 21 days the dosage was changed to 2 cc. of the female hormone plus 1 cc. of the male hormone.

The comb growth and body weight records for the time of injection and one month after injections were interrupted are tabulated below. The headfurnishings became red and turgid during the injections: after these were discontinued regression with accompanying pale and shrivelled appearance took place

DATE	COMB		WEIGHT	
	L.	D.		
	cm.	*cm.*	*grams*	
1-10-30	4.2	2.2	1724	
1-12-30				Injections begun
1-17-30	4.6	2.4	1699	
1-24-30	5.5	2.8	1645	
1-31-30	5.8	3.3	1670	
2- 7-30	6.2	3.8	1678	
2-13-30	6.6	3.7	1655	Injections ceased
3-13-30	5.4	2.6	1733	

The incoming plumage was female in anterior breast, back and saddle. Records of the modified feathers were made one month after plucking. The findings here are comparable in every way to the observations made in experiments I and II, where the female and male hormones were injected simultaneously. *There are, then, no indications of a modification of the hormones when these are mixed "in vitro" for 48 hours.*

Experiment V. *Female and male hormones injected on alternate days.* Capon 29–41 received injections of 1 cc. of the male hormone every second day. The female hormone was injected on the alternate day. The dosage of the female hormone was 1.8 cc. for the first 6 injections and 2.5 cc. during the succeeding 11 injections.

The headfurnishings showed slight growth during the time of injection: they be-

came pink in color and rather smooth but by no means red or turgid. It is of course to be noted that the quantity of male hormone is reduced to one-half compared to the previous experiments and similarly for the female hormone.

The figures for comb growth and body weight during injections and one month after these were discontinued are tabulated in the following. The regression of the slight growth obtained in the headfurnishings when the injections were discontinued is evident.

DATE	COMB		WEIGHT	
	L.	D.		
	cm.	*cm.*	*grams*	
1-10-30	4.1	2.0	1998	Injections begun
1-17-30	4.4	2.2	1962	
1-24-30	4.5	2.1	1866	
1-31-30	4.9	2.5	1939	
2- 7-30	4.8	2.4	1958	
2-13-30	4.9	2.6	1982	Injections ceased
3-13-30	4.3	2.3	2045	

The plumage changes were studied two months after plucking when the regenerating feathers were almost completely developed. The replacing feathering in the four regions seemed entirely male but careful study showed faint female barring in the saddle. This region alone showed any indication of response to the female hormone.

It is clear from these findings that there is no intensification of the action of the one sex hormone through the presence of the other in the organism.

Discussion. The experiments described offer no indications of an antagonism between the chemically prepared sex hormones either in vitro or in the organism. The simultaneous presence of the female and male hormones causes the appearance in the gonadless bird of certain secondary sex characters pertaining to each sex: female plumage and male headfurnishings.

The presence in the organism of the one sex hormone does not intensify the action of the other—a subthreshold value of the female hormone is not brought to an effective concentration through the administration of the male hormone and vice versa. (expt. V.) These findings are reasonable when related to the conception of a qualitative chemical difference in the hormones. The male and female hormones are obviously different substances which do not interact chemically.

The biological implications of the term "antagonism" merit some discussion. They involve: 1. Modifications induced by the one sex hormone in the opposite sex gonad. This point is not dealt with here.

2. A direct action of the two hormones upon each other, e.g., neutralization, such an antagonism is excluded for the preparations used.

3. A simultaneous and differing action of both sex hormones upon one and the same character. This conception requires that a given character, or embryonic rudiment, may be positively affected in different directions by the two hormones.

Is such demonstrably the case for any sex character in normal development? The indifferent germ cells in the fowl appear to develop in either the male or female direction according to their location in medulla or cortex (Domm, 1929, p. 183). Since both hormones are presumably elaborated simultaneously in a bi-sexual gonad, the determinant factor would appear to rest with the proximity of the germ cells at a critical period to the medullary or cortical tissues, differentiation occurring at the point of maximum hormone concentration.

The plumage is hormone conditioned in the female and independent in the male. The spurs (Domm, 1929a, pp. 466–67) are hormone conditioned in the female, independent in the male. The Wolffian ducts are positively determined in the male, neutral in the female. The oviduct, positively determined in the female, is lacking in the male. Thus none of these characters may be simultaneously affected by the two sex hormones.

The headfurnishings differ from the secondary sex characters listed since they are positively determined in each sex. The response elicited, however, is of the same order, i.e., towards growth, in either sex, and differs only in degree (Hardesty, 1930). Therefore, as the headfurnishings are similarly affected by the hormones secreted by the gonads, they furnish no criterion of hormone antagonism.

The female hormone determines the appearance of female feathering and causes no diminution in size of the male comb. Since the female hormone in the normal bird stimulates comb growth to some extent, an inhibiting effect on the established male headfurnishings is scarcely to be expected.

In conclusion it may be noted that the effects resulting from the simultaneous injection of the two chemically prepared sex hormones agree with other findings already recorded in the fowl.

Thus Domm has shown in the brown leghorn (Domm, 1927) that following ovariotomy the female develops male plumage and male headfurnishings. Male plumage is characteristic of the gonadectomised bird of either sex but male headfurnishings develop only in the presence of the male hormone, secreted here by the medullary component of the activated right gonad. Subsequently, in a large number of poulards there occurred a reversion to female plumage, while the comb, etc., remained male.

This female feathering is conditioned by the secretions of the cortical elements in the right gonad which had attained sufficient development in turn (Domm, 1929; Gray, 1930).

In these cases the same conditions prevail as experimentally realized

by the simultaneous injection of the two sex hormones and the same behaviour of the secondary sex characters is realized, the headfurnishings are male, the plumage is female.

SUMMARY

1. The effects of combined injections of the female and male hormones and of alternate injections on successive days were studied in adult capons.

2. The birds receiving the simultaneous injections developed female plumage and male headfurnishings.

3. The same results were obtained when the hormones were mixed "in vitro" for twenty-four hours and forty-eight hours respectively previous to injection.

4. When the hormones were administered in the same concentration but on alternate days female plumage was not obtained and the headfurnishings showed only very slight growth.

5. There are no indications of an antagonism between the chemically prepared sex hormones with the preparations used in the adult capon, neither is there an intensification of the action of one hormone in the presence of the other.

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ON THE NATURE OF THE NERVE IMPULSE

I. The Effect of Carbon Monoxide on Medullated Nerve

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The object of this series of papers is to elucidate more clearly the chemical nature of the processes which cause and accompany the propagated impulse in nerve, giving particular emphasis to phenomena of oxidation and reduction. Warburg (1928) has shown that oxygen used in cellular respiration has first to be activated by a respiratory enzyme which appears to be universally present in cells. Although the function of this catalyst, that of oxygen activation, is presumably the same in all cells, its chemical properties may vary considerably in different types of cells. This is accounted for by Warburg as due to slightly different chemical configurations of the heavy metal, hemin-like active centers, an assumption that is justified, perhaps, by the differing catalytic properties of hemin when united with various substrata, such as nicotine, pyridine, etc. In general, however, this heavy metal complex can be poisoned by carbon monoxide, cyanide and hydrogen sulphide. Of these, perhaps the most specific—certainly the most important in the development of the Warburg theory—is carbon monoxide. The brilliant researches of Warburg and Negelein (1929) in which they make use of the light sensitivity of the carbon monoxide complex in determining the absolute absorption spectrum of the respiratory enzyme strikingly demonstrates this fact.

Inferential evidence has been accumulating that the propagation of the nerve impulse depends in some way on oxidations. This has been derived largely from the fact that conduction does persist for a time in nitrogen and that the nerve develops an oxygen debt during anaerobiosis; similar inferences may be drawn from the relation between the initial and delayed heat productions of nerve (Gerard, 1927). Based on the fact that stimulation of nerves during anaerobiosis produces no extra carbon dioxide (Schmitt, 1929), I was led to the hypothesis that the action potential may require union of oxygen with substances in the nerve, rather than complete oxidation. Since the oxygen was intramolecular, it must have been activated in the usual sense of this term. This hypothesis seems valid for anaerobic conduction but no direct evidence was available as to the oxidative nature of the impulse during aerobic conduction.

The obvious starting point for these investigations was to determine whether the oxygen required for conduction has first to be activated, and if so, then whether the respiratory enzyme acts as catalyst for the reaction. For reasons referred to above, the use of carbon monoxide seemed to be the most promising experimentally. The effect of cyanides and of hydrogen sulphide both upon the respiration and upon the action potential of medullated nerves will be reported separately in papers to follow shortly.

1. *The effect of carbon monoxide on nerve metabolism.* For the study of the effect of carbon monoxide on the oxygen uptake of resting nerves the Warburg manometric technique was employed. Small vessels,[1] of about 4 cc. capacity, with an inset for alkali were used. The method of procedure usually followed has been to remove and weigh five nerves (R. pipiens) and suspend them in the vessel containing 0.2 to 0.3 cc. of Ringer solution, the inset containing 0.2 cc. N/10 NaOH. A second vessel contained the five companion nerves similarly treated, while a third vessel served as a control. The carbon monoxide was generated by dropping formic acid into hot, concentrated sulphuric acid. The gas was passed into a gasometer and the proper admixture with oxygen made. The composition of the mixture was determined by analysis and the gas then passed into the experimental vessel. After following the rate of oxygen uptake for an hour or so in the dark, the vessel containing the carbon monoxide-oxygen mixture was illuminated from above by means of an arc light. Then, in another hour or so, the illumination was discontinued and the respiration followed for another period of time in the dark. Invariably, light caused a decrease of inhibition as is shown in figure 1. It is noteworthy that this effect of light is confined sharply to the period of illumination; there is little after-effect.

That the catalytic mechanism of respiration in nerve is similar to that in other tissues is further shown by the fact that the value of the constant K, in Warburg's expression

$$K = \frac{n}{1-n} \cdot \frac{CO}{O_2}$$

in which n is the residual respiration of the nerve in carbon monoxide, approximates closely to that found by Warburg (1927) and Keilin (1929). Table 1 summarizes some of the experiments illustrating this point. It may be seen that the value of K in the absence of light, which averages 8.6, is raised to 21.2 by illumination, an increase of but two and one-half fold. Obviously, however, the conditions under which the nerves were illuminated were not such as to produce a maximum light effect. The vessels were not designed for this purpose and only a small part of the light striking the vessel reached the nerves. Furthermore, since the nerves were suspended in solutions the vessel had to be shaken, hence it was illuminated only in the

[1] The manometers and vessels were very kindly loaned by Dr. Ethel Ronzoni.

central portion of its excursion. A vessel specially designed for this purpose and containing electrodes to lead off the action potential to the cathode ray oscillograph is now available and experiments involving these simultaneous measurements will be reported in the near future.

2. *The effect of carbon monoxide on the action potential of nerve.*[2] For the measurement of the action potential of nerves under the influence of carbon monoxide, a special hard rubber chamber was constructed. The

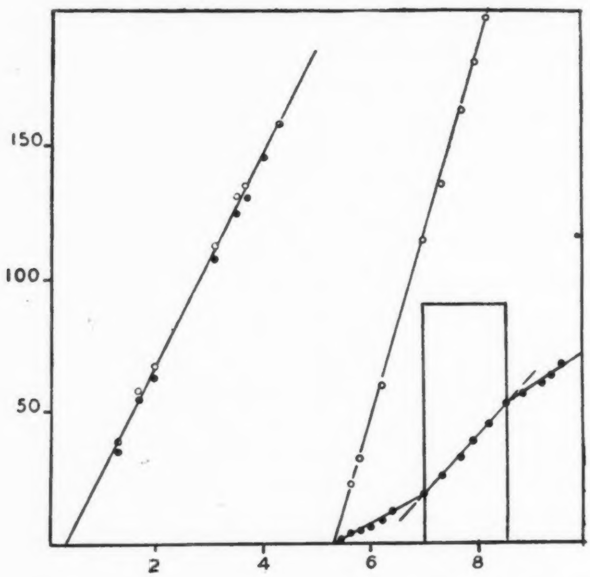

Fig. 1. Effect of carbon monoxide on oxygen uptake of resting frog nerves. Ordinates represent oxygen uptake in cu. mm. per gram nerve; abscissae, time in hours. Circles represent uptake of control nerves; points, that of experimental nerves. First, the oxygen uptake of each set was measured in air and shown to coincide. Then experimental nerves were exposed to a mixture containing 96.5 per cent carbon monoxide and 3.5 per cent oxygen. Period of illumination represented by rectangle

electrodes are of heavy silver wire and a depression in the chamber makes it possible to immerse the nerves in solutions for a portion of their lengths if so desired. The two nerve chambers may be connected by means of a

[2] This portion of the work was made possible by a grant to Washington University by the Rockefeller Foundation and was carried out in part at the Marine Biological Laboratory, Woods Hole, Mass. I am indebted also to Mr. Otto H. A. Schmitt for invaluable assistance in the construction of the oscillograph.

small tube or they may be used independently as was done in most of the experiments now under consideration. Four stopcocks permit rapid changing of gases while terminal water manometers serve to indicate possible leaks. Strips of moistened filter paper placed along the sides of the chambers and the Ringer solution in the wells prevent drying of the nerves. Under these conditions nerves have maintained their irritability as long as three days. In all experiments on carbon monoxide the nerves were laid over the electrodes without dipping into the Ringer solution in the well. This was necessary for the purposes of illumination. The conduction distance was short, preventing the separation of action potential waves and to obtain as nearly as possible a picture of the true axon action potential.

When pure carbon monoxide or nitrogen was used these gases were passed over glowing copper through sealed tubes. It was found that mixtures in which the percentage ratio of carbon monoxide to oxygen was fifteen or less have little effect on the height of the action potential. Any appreciable decreases which obtained under these conditions usually required hours to manifest themselves. When the percentage ratio reached twenty-five to thirty, however, extinction was complete in one to three hours. The length of time required for the extinction of the action potential in pure carbon monoxide is the same as that in pure nitrogen: from one to two hours in these experiments. As in the case of nitrogen asphyxiation, a second or third asphyxiation in pure carbon monoxide causes extinction in much shorter periods of time than is required for the first. Similarly, after recovery from asphyxiation in pure carbon monoxide, mixtures containing oxygen in amounts such as would normally have little effect on the action potential now cause rapid extinction.

TABLE 1

DATE	TEMPERATURE	COMPOSITION OF GAS		PER CENT INHIBITION		K.	
		Per cent O_2	Per cent CO	Dark	Light	Dark	Light
1930	°C.						
April 17	19.9	5.8	94.2	40		25	
April 18	19.9	3.2	95.0	78	59	8.5	21
April 21	20.5	3.5	96.5	83	71	5.5	11
May 28	22.8	4.0	96.0	70	58	10.5	17
May 29	22.4	9.3	90.7	52		8.9	
May 30	21.0	13.8	83.7	66	53	13	36
Average						8.6	21.2

In measuring the height of the action potential as recorded on the Braun

tube, a measuring device accurate to a fraction of a millimeter was used. The maximum height so measured was recorded and a photograph of a single deflection then made. These two methods of measurement yield identical results hence in most experiments herein recorded, some of which required as many as two to three hundred measurements, readings were made with the measuring device only and were not checked photographically.

By far the most striking result of these experiments is the fact that nerves that are failing in a carbon monoxide-oxygen mixture, when exposed to light, return to normal activity. In order to facilitate the illumination of as many of the fibers as possible, relatively small nerves were used and the illumination to which they were subjected was made very intense. The most satisfactory results were obtained by the use of Ever-ready "B" carbons with a current of 25 amperes across the arc. The light was filtered through a $2\frac{1}{2}$ per cent solution of copper sulphate and concentrated by means of lenses. In this way a very intense beam was obtained whose spectral distribution extended between 3000 and 6000 Å.U. with a maximum in the region of 4000 Å.U. The light was so arranged that the nerves in both chambers were illuminated to the same degree. Temperatures were determined by a thermometer placed midway between the two chambers.

Figure 2 illustrates a typical experiment. One nerve had been placed in pure carbon monoxide, the other in pure nitrogen. Total extinction occurred in both in 100 minutes, and, although both nerves were illuminated during the interval of their failure, no light effect appeared in either. Recovery was prompt on the admission of oxygen, at *A*. Some time after recovery, at *B*, nitrogen was again passed into the chamber that previously contained it but into the other chamber was passed a mixture containing 96 per cent carbon monoxide and 4 per cent oxygen. Extinction of the action potential required but 60 minutes in nitrogen and illumination again had no effect. Extinction in the carbon monoxide-oxygen mixture also was quite rapid but, as shown in figure 2, illumination caused an abrupt rise in the height of the action potential. This striking effect of light occurred each time the nerve was illuminated; seven times in this experiment. It is to be noted that in most cases the curve representing the effect of light is a fairly symmetrical one; the rise to a maximum during illumination requires roughly the same duration of time as the fall to the original extinction curve after illumination is stopped. Admission of oxygen, at *C*, caused a partial recovery.

That this effect is that of light and not one of temperature rise or of photo-oxidation is indicated by results recorded in figure 3. In this case one nerve was immersed in a gas mixture containing 97.9 per cent carbon monoxide and 2.1 per cent oxygen, the other nerve in a mixture containing 99 per cent nitrogen and 1 per cent oxygen. Extinction was complete in

both nerves in 170 minutes. During the period of failure, however, three exposures to light produced three distinct rises in the height of the action

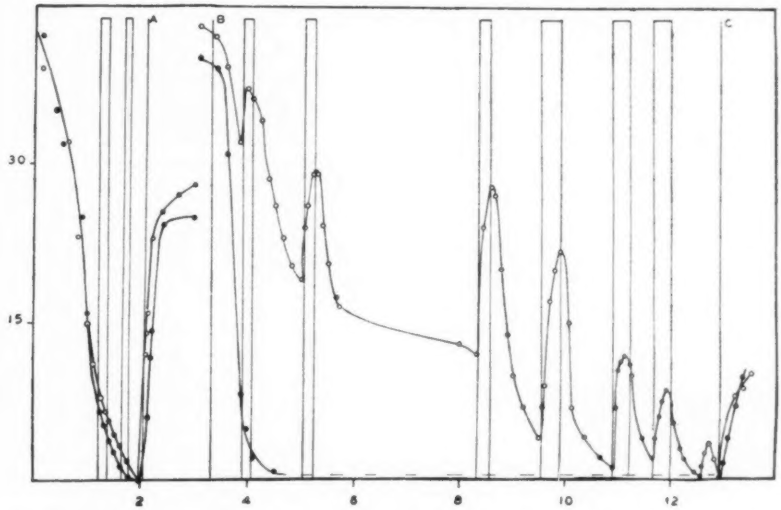

Fig. 2. Ordinates, deflection of action potential in millimeters on the Braun tube; abscissae, time in hours. Potentials of nitrogen-treated nerve represented by points, those of carbon monoxide-treated nerve by circles. The periods of illumination are marked by rectangles. Further description in text.

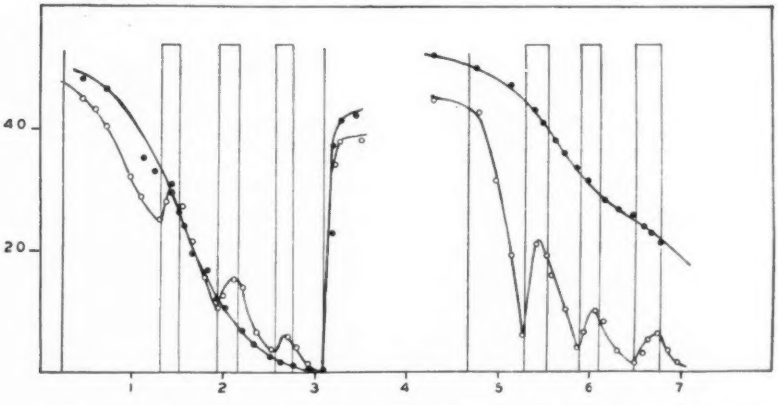

Fig. 3. Same as preceding. Note absence of light effect in nerve in nitrogen-oxygen mixture.

potential in the nerve treated with carbon monoxide but had no effect on the nerve in nitrogen. Recovery of both nerves was prompt in oxygen. Then after recovery the experiment was repeated, this time, however, the mixtures contained 96.3 per cent carbon monoxide and 3.7 per cent oxygen, and 96.3 per cent nitrogen and 3.7 per cent oxygen, respectively. Extinction of the action potential in the nerve in the nitrogen mixture was very slow and never complete, while extinction in that in the carbon monoxide mixture was rapid as usual. Three periods of illumination again

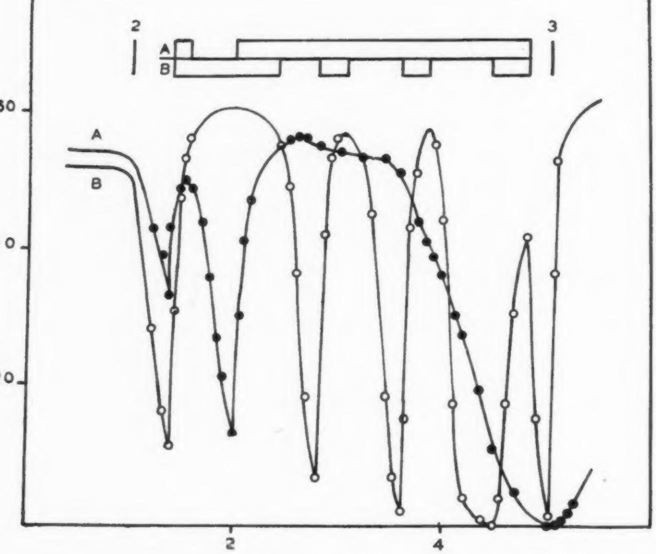

Fig. 4. Effect of continuous illumination of nerves in a carbon monoxide mixture. Illumination of nerves *A* and *B* represented by the horizontal rectangles similarly designated. Coördinates as before.

caused three rises in the height of action potential of the nerve in the carbon monoxide while the nerve in the nitrogen mixture remained unaffected by light.

Having demonstrated that light may produce a *temporary* return of action potentials which are falling in carbon monoxide-oxygen mixtures, we next set out to determine how long this effect of light could be made to last. Figure 4 illustrates an experiment planned to answer this question. A mixture containing 98.1 per cent carbon monoxide and 1.9 per cent oxygen was passed into both chambers, at *2*, with the result that both nerves

began to fail along a fairly steep curve. Equal illumination of both nerves caused the height of the action potential to rise in the nerve of each side; indeed, in one case the height exceeded somewhat the original value. This condition remained as long as illumination was continued, in this case 40 minutes. As shown in the figure, illumination may cause the action potential to rise from a very low level, to or above the normal level and maintain it there for as long as two hours. It is our impression that the ultimate slow drop in the action potential that occurred in this case was due to certain unfavorable conditions caused by the long continued operation of the high amperage arc light rather than to a failure of light to prevent the inhibition. This impression is strengthened by the fact that the nerve

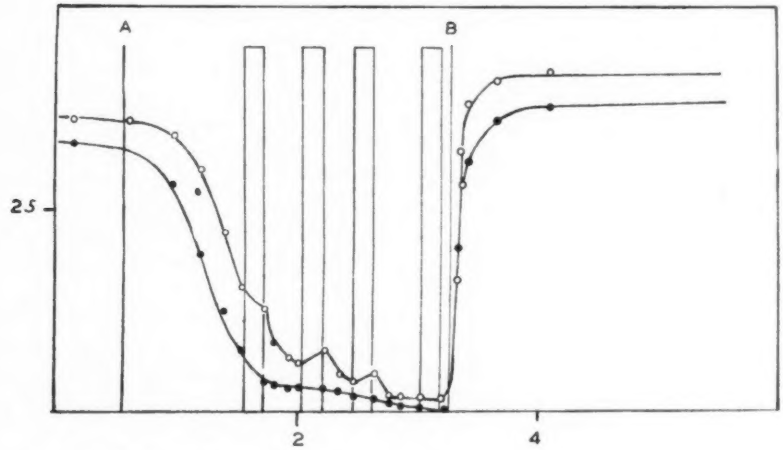

Fig. 5. Effect of illumination of nerve in pure (?) carbon monoxide and in pure nitrogen. Action potential of nerve in nitrogen represented by points, those in carbon monoxide by circles.

when illuminated for over three hours continuously, failed to recover to nearly the extent of the companion nerve upon admission of oxygen. Figure 4 also strikingly illustrates the extent to which illumination may, under favorable circumstances, cause the return of the action potential to or above the normal level. In this experiment, although the action potential had disappeared completely for a time, illumination caused a return practically to normal level. Admission of oxygen caused prompt recovery. To obtain effects of light such as those illustrated in figure 4, it appears probable that certain optimum conditions must prevail. Of these, perhaps the most important are the partial pressure of carbon monoxide and the intensity of the light.

Warburg and Negelein (1929) have recently investigated the absolute absorption spectrum of the respiratory enzyme in yeast. They find that a maximum exists in the region of 4300 Å.U. Similarly, we hope to determine the region of greatest light susceptibility of nerve poisoned in carbon monoxide. Using Corning filters, it appears from preliminary experiments that a blue filter transmitting light with wave lengths from 3800–4800 Å.U. with a maximum at 4400 Å.U. is just as effective as the unfiltered arc light notwithstanding the reduced intensity. With other filters, however, transmitting wave lengths up to 7000 Å.U., striking effects were also obtained although neither the intensity nor the heating effect of the long wave lengths was controlled.

Although the illumination of nerves that are failing in nitrogen has no effect on the height of the action potential, some evidence was found that illumination of nerves failing in presumably pure carbon monoxide does produce small increases in its height. This is illustrated by the curves in figure 5. Similar results were obtained in nerve-muscle preparations by Schmitt and Beck (in press), using the threshold method for the determination of nerve irritability. The phenomenon did not occur consistently and it is possible that the explanation is to be found in the fact that very small traces of oxygen might conceivably have been present in some of the experiments such as the one just mentioned, and that these traces sufficed to produce the effect. This seems the most obvious explanation, though further consideration is being given the matter.

Discussion. The experiments upon the metabolism and those upon the electric response of nerve under the influence of carbon monoxide reported in this paper were necessarily done separately and on different nerves. It is our hope, in further experiments now in progress, to record with the oscillograph the electric responses of nerves and measurements of their metabolism simultaneously, for it is clear that final and convincing evidence as to the chemical nature of the impulse can come only from such combination of methods. Nevertheless, certain possibilities are suggested by the present work which warrant brief discussion at this time. In the first place, it seems clear from results of the experimental work of Warburg quoted above and from those of the experiments herein reported that the action potential in nerve is an oxidation phenomenon and that the oxygen used in the process requires activation by the heavy metal catalytic complex which is poisoned by carbon monoxide. For anaerobic conduction the source of oxygen is presumably intramolecular, from substances which in the past have been called oxidative reserves. However, it now appears likely that the oxygen required for the action potential, *whether aerobic or anaerobic,* must originally have been activated by the heavy metal catalyst This is indicated by the fact that after the action potential has *completely* disappeared in a carbon monoxide-oxygen mixture which contains enough

oxygen to maintain the resting metabolism at perhaps a quarter of its normal rate, it is still possible to restore the action potential rapidly and to its full height by illumination, as figure 4 illustrates. It seems highly improbable, however, that the catalyst is required in the final chain of events the immediate result of which is the production of the electric action potential, for nerves do not fail more rapidly in pure carbon monoxide than in pure nitrogen. It would seem rather that the catalyst simply provides the source of activated oxygen; it is the breakdown of substances thus oxidized or partially oxidized which causes the electric potential.

The important questions now present themselves: what is the mechanism of this chain reaction and what are the substances the oxidation of which is so necessary for the production of the action potential? If, for the present, we assume that our experiments on metabolism and those on electric response are comparable, it follows that the oxidative reactions which are involved in producing the action potential are not exactly similar to those involved in the metabolism of resting nerves, though not independent of them. For, although a given mixture of carbon monoxide and oxygen may render the nerve completely non-irritable, it may still permit from 15 to 25 per cent of the normal respiratory rate to proceed. Similar results have been obtained with cyanides by Schmitt and Schmitt (paper to follow). Nor is there correspondence between the time necessary for the return of the action potential after asphyxial failure and the payment of the oxygen debt, the former occurring usually after only a few minutes, while the latter may require from thirty minutes to an hour. A further difference is found in the fact that the effect of light on the respiration of nerve in carbon monoxide-oxygen mixtures is closely confined to the period of illumination whereas a definite after-effect of the light on the action potential is observed which may last for thirty minutes or more. Finally, it is perhaps not insignificant that when a nerve had almost completely failed in a carbon monoxide-oxygen mixture, the curve produced by illumination (see fig. 4) was usually quite symmetrical. The curve was produced by illuminating the nerve until the action potential had just reached a maximum; at this time illumination was discontinued. The action potential then fell off along a regular curve. Had illumination been continued after maximum potential height had been obtained it would doubtless have continued at its high value. The after effect of light (i.e., the time required after stopping the illumination for the action potential to return to zero or to the original extinction curve) was found to be fairly constant from experiment to experiment and to amount to 25 or 30 minutes. In this connection a common experience may be recalled, namely, that exposure of an asphyxiated nerve to oxygen for only a short time followed by re-exposure to nitrogen produces much more rapid extinction the second time than in the initial asphyxiation. Fröhlich (1904) found that the sec-

ond extinction required roughly 30 minutes, a value that agrees with that of our own experience.

These facts suggest a possible interpretation of the so-called "oxidative reserve" somewhat different from that usually inferred in the literature. Assuming physical conditions, proper ion ratios, etc., to remain constant, it would seem that the production and propagation of a maximum action potential is associated with the presence of a *rather definite amount* of some oxidized substance. This amount is perhaps small in comparison with that which corresponds to the total oxidative reserve which is measured by the oxygen debt. It is specifically required for the propagation of the impulse aerobically and in this sense should not be called a reserve, although it may appear to act as such when oxygen is withdrawn. Whether this substance is a sort of oxygen carrier[3] to convey the activated oxygen to certain molecules in the irritable mechanism, or is itself actually the final substance to be oxidized, oriented, perhaps, at certain critical interfaces, remains to be determined. For the ultimate solution of these questions much experimentation will be required and the views herein expressed are offered merely as a tentative hypothesis pending further evidence.

SUMMARY

1. The respiration of nerve is inhibited in the dark by carbon monoxide-oxygen mixtures. This inhibition may be fairly complete, the degree depending on the partial pressure of the carbon monoxide and upon conditions of diffusion in the nerve. Light markedly decreases this inhibition, the decrease stopping when the illumination is discontinued.

2. The maximum height of the action potential as measured by the cathode ray oscillograph is reduced in carbon monoxide-oxygen mixtures in the dark, and ultimately decreases to zero, the time required for extinction depending on the partial pressure of the carbon monoxide. Intense illumination of the nerve during the period of the failure causes the height of the action potential to rise to, and in some cases to exceed somewhat the normal level. When illumination is discontinued, the action potential does not drop immediately to the original extinction curve, but does so gradually after a period of time. This period of after-effect of light is of fairly constant duration and usually lasts 25 to 30 minutes.

3. These facts are interpreted to mean that the action potential whether aerobic or anaerobic requires an oxidation or oxygenation of substances in nerve and that activation of the oxygen by a respiratory enzyme similar to

[3] It is possible that the substance cytochrome may function in the capacity of an oxygen carrier in this system. Although certain conditions inherent in the structure of nerve make a search for cytochrome difficult, we are at present undertaking it in both medullated and non-medullated nerves with the aid of a microspectroscope.

that of Warburg is essential. The mechanism of this oxidation system is briefly discussed.

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THE ANTERIOR LOBE AND MENSTRUATION

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It was shown by Corner and again by Edgar Allen that menstruation may proceed, at least for a limited period, without ovulation or the formation of the corpus luteum. This point has now been corroborated in a long series of observations, especially on living animals, in the Carnegie monkey colony, covering several years and involving scores of laparotomies, several hundred rectal-bimanual palpations of the normal living animals, as well as several autopsies. It has been found, indeed, that whole seasons pass, namely, the summer months (the non-breeding season) in which uterine bleeding proceeds, even though more or less regularly, almost if not entirely without ovulation and with only slight waves of growth and decline of ovaries and uterus,—a conclusion already arrived at in 1905 by Marie A. van Hewerden in her brilliant doctor's thesis at Utrecht.

It is clear, then, that there is something radically wrong with the prevailing or "modern" gynecological theory of the menstrual process which presupposes ovulation and makes the degeneration of the corpus luteum responsible for the uterine bleeding. The senior author of this communication has previously emphasized the inadequacy of the theory which sets up the "absence of" something as the causative factor of a far-reaching physiological process. In order to get a new point of departure it was reasoned that the cause of menstruation is an *active* one, probably hormonal and that the facts require us to look outside the ovaries for the source of the stimulus. The prominent rôle which the logic of Hofbauer and the experimental work of Smith and Engle and of Ascheim and Zondek have definitely assigned to the anterior lobe of the hypophysis in the reproductive functions of the body (a conclusion adumbrated by much clinical experience and some experimentation in the past) led naturally to the selection of that organ as the one responsible also for bleeding. We believe to have established the correctness of this presumption in a series of experiments involving a dozen or more hypophysectomies (Doctor

Firor) and the use of ovarian and hypophyseal extracts[1] (Doctor Geiling[2] and E. R. Squibb & Sons[3]). It is the object of this communication to report the result of some of these experiments, for it is believed that they place the menstrual phenomenon upon a scientific footing.

Edgar Allen and lately his assistant Maddux, also Morrell and associates, have shown that when the Allen-Doisy hormone (ovarian extract; Amniotin, Squibb) is injected into a spayed macaque a bleeding from the uterus results. In our own experiments on normal or castrated females (the action is the same in either case) the bleeding begins, typically, towards the end of a 6 to 8 days' administration of the hormone and continues 8 to 10 or even 20 days. The long duration of the bleeding is a characteristic effect of these injections.[4] Allen and co-workers have used adolescent females and we have repeated their experiments a number of times on castrated females, on adults a long time amenorrheic, and on babies only a year old, both intact and castrated. We regard the experi-

[1] Thanks are due to Greenwald Incorporated, packers of Baltimore, for their kindness and coöperation in all of the gland work conducted by the Department of Pharmacology.

[2] Methods of preparing the extracts of the anterior pituitary.

a. Doctor Geiling's alkaline extract: This was prepared from *fresh* glands according to a modification of the method outlined by Evans and Simpson. Less alkali was used and the alkaline material was allowed to stand in the ice-box over night before the pH was adjusted to 7.4. The resultant preparation was clear and potent.

b. Doctor Geiling's acid extract: Fresh glands were finely ground with sand, three volumes of distilled water was added with sufficient acetic acid to give a pH of about 2.0 (approximately 0.25 per cent of acetic acid). The acidified material was placed in an ice-box over night and centrifuged the following morning, the clear extracts were then kept on ice as they were being used, new batches being made up every 4 or 5 days.

c. The alkaline extract kindly made up for us by E. R. Squibb & Son: The urine of pregnant women was acidified with acetic acid to a pH of approximately 4.6, filtered and precipitated with $4\frac{1}{2}$ volumes of 95 per cent alcohol. The solids were removed by filtration and centrifuging and washed thoroughly with ether and dried. This precipitate was extracted with water and any insoluble material centrifuged out. This process is repeated twice more, resulting in a clear aqueous extract which contains most of the active principle present in the original urine. This preparation is in the experimental stage and not yet commercially available.

[3] Work with the Allen-Doisy hormone was made possible through the coöperation of the Department of Biological Research of E. R. Squibb & Son, to whom our thanks are due.

[4] Morrell, Powers, Varley and deFrates found a latent period in the appearance of the bleeding amounting to 10 to 12 days and a duration of the bleeding not greatly in excess of the usual menses. The difference in their results and our own seems to be due to the difference in the methods employed in searching for red blood cells. We call the presence of one or two red blood cells in 0.1 cmm. of the lavage positive. We have, however, nevertheless in some cases met with a delay in the appearance of blood.

ments on the babies between 1300 and 1700 grams in weight as particularly conclusive. These yearlings may be said to have the "equivalent age" of a three to four year old girl. We give examples:

No. 91. Normal yearling of 1330 grams; 375 rat units of Amniotin Squibb administered in increasing doses over a period of 10 days. Several red blood cells in the vaginal lavage days 3, 4, and 6 of the injections; again 9, 10, and 11; considerable bleeding days 13 to 18, microscopic in amount days 19 to 24—a continuous bleeding for 16 days in a mere baby!

No. 101. Yearling of 1390 grams. Control: Brain exposed, retracted, replaced, hypophysis left intact; 370 rat units of Amniotin Squibb administered over a period of 8 days. Microscopic bleeding from day 6 of injections on, continuing for 16 days, macroscopic in amount (tinge) on day 12 after the first dose.

No. 99. Yearling of 1610 grams; completely ovariectomized; 375 rat units. Amniotin Squibb administered in increasing dosage over a period of 10 days. Days 3 4, and 5, a few red blood cells in lavage; days 10 to 27 more of them, in very considerable numbers days 16–23—a continuous bleeding for 20 days in a spayed baby monkey following the injection of Allen-Doisy hormone!

The question now arises: does the ovarian hormone of Allen and Doisy act directly upon the uterine mucosa, rendering it permeable to red blood cells—or does it act through the hypophysis? The following experiment is so clear-cut that there is no room for doubt as to the correct answer:

No. 125. Yearling 1760 grams in weight. Hypophysectomized June 5, 1930. Beginning June 30, 495 (almost 500) rat units of Amniotin Squibb injected over a period of eight days. Absolutely no bleeding resulted, whereas the three controls bled for 16, 16, and 20 days respectively. In all four subjects the vulva was somewhat swollen. No. 125 remained in excellent condition and in its actions in the paddock differed in no wise from the normal babies of the same age. Neither did the half-dozen hypophysectomized male babies, housed with the others, betray their loss of the important gland.

Such is the indirect evidence from the injection of ovarian hormone. What is the effect of the administration of hypophyseal substance? This question we shall first answer with the hypophysectomized no. 125 just alluded to:

A month after no. 125 had received her last dose of Amniotin Squibb she received intraperitoneally 41.5 cc. of alkaline extract (Squibb) over a period of ten days. On days 4 to 8 inclusive of the injections red blood cells were recovered in the vaginal washings in very small numbers, from one to six or seven in a sample, nevertheless quite sufficient to declare the effect positive.

This positive result (bleeding) with extract of the anterior lobe on a hypophysectomized female baby, previously negative to massive doses of Amniotin Squibb, is paralleled by the results of treatment with anterior lobe extract of the fully adult hypophysectomized female, no. 20.

No. 20. Female no. 20 is said to have had a baby in a zoölogical park about five years ago, before she was added to the Carnegie colony. She is peculiar in that she

usually, though not always, refused the male at any part of the menstrual cycle, yet she menstruated with considerable regularity at intervals averaging 28.1 days, and she also ovulated, as determined by two laparotomies and later by rectal-bimanual palpation. July 30 last she was hypophysectomized, whether completely or not remains to be seen. It was nevertheless possible, with alkaline anterior lobe extract (Squibb) to elicit slight bleeding. Injections of 2, 3, 4, 5, 6, 4, 3 cc. respectively per day (the last day in a single intraperitoneal dose, on the other days distributed over three doses) were followed by very slight bleeding on day 6, that is, the day the dose dropped to a single injection of 3 cc. Without interruption the injections were continued with daily doses of 4, 5, 9, 15, 15 cc. respectively, distributed over three injections daily. The day following the last injections (the animal now having received a total of 66 cc.) a more considerable bleeding occurred. Nine days after the last injection an increase in the size of the left ovary ("medium, round") was definitely determined by palpation, but no change in the uterus was apparent to the touch.

The same extract elicited bleeding in no. 69, an emaciated old multipara that had had two still-births in the Carnegie colony, the last one May 1, 1930. She had since been suffering from amenorrhea, with infantile uterus and ovaries. In this case the bleeding began day 5 of the 10 days' injections of a total of 62 cc. of extract and continued for 15 days. No increase whatever was induced in the size of ovaries or uterus.

The foregoing experiments had been preceded by less conclusive ones on normal babies and other amenorrheic females, including several decrepit adolescents and adults. The results are corroborative of the more crucial experiments detailed above and are of interest in connection with the evidence presented in this paper:

No. 91. Yearling baby of 1330 grams. February 17, three triturated anterior lobes of adult castrated male pigs were injected sterile by means of a trochar into the peritoneal cavity. Considerable bleeding occurred the fourth and fifth days thereafter.

No. 31, an adolescent female, on June 10, 11, 12, and 13 received daily, by intramuscular transplantation, similar anterior lobes, one daily. Bleeding occurred June 17, 18, and 19.

No. 85, treated similarly to the last, bled lightly days 2 to 8 after the last transplant.

Bleeding also occurred in amenorrheic and sick adolescent females as follows:

No. 82. On days 7 to 16 following the last of 8 days' intraperitoneal administration (5 cc. daily) of urine from a woman six months pregnant.

No. 82 again. On days 5 to 8 following the intraperitoneal administration of acid extract (Geiling) of anterior lobe, no. 82 bled again.

No. 83. On days 6 to 10 (killed on day 10) after a 13-day intraperitoneal administration of alkaline extract (Geiling). A little blood was recovered on days 5, 9, and 10 of the injections.

No. 86. On days 1 to 10 following a 7-day injection of acid extract (Geiling).

No. 83 remained negative to acid extract in lesser dosage, no. 85 to alkaline extract.

Two other hypophysectomized females are available for study. Each received 430 rat units of Amniotin Squibb in increasing dosages over a period of 7 days beginning the day after the operation, with the following results:

No. 36. A multipara that had given birth to and nursed a baby the preceding year and later suffered from a serious illness from which she completely recovered. On days 16 and 17 there were 2 red blood cells in a 0.1 cmm. sample of the vaginal lavage. Twenty-seven days later a few red blood cells again appeared, which marked the end of an apparently spontaneous cycle. No. 94, an adolescent, showed blood days 15 to 22 after the first injection and a month later for one day (August 21) had a few red blood cells appearing spontaneously in the vaginal contents. The vaginal lavage also betokened a cycle of vaginal desquamation.

It is apparent, therefore, that a little anterior lobe had been left in the sella turcica of these two females. The bleeding in these cases was most minute and would have escaped detection except for the meticulous method employed to demonstrate free blood. Hence the effect of the treatment may be said to have been almost zero. The operation had obliterated the response to Amniotin Squibb almost to a vanishing point in spite of the fact that injections were begun the day after the operation. It is not illogical to postulate the survival of a portion of the gland—a point which the eventual autopsy will definitely decide. They were, however, subjected to another course of treatment with Amniotin Squibb beginning August 26, as detailed below:

No. 36 received 410 rat units in seven days in doses increasing from 20 to 90 per day, three injections daily except the first day. Bleeding resulted which began day 6 of the injections and continued for 12 days, being very minute except the first two days following the last injection, when the bleeding was sufficient to tinge very lightly the vaginal lavage. The effect was typical of Amniotin treatment though much lighter than in a normal animal. Vaginal desquamation rose with the injections to 35 per cent but dropped to zero immediately on the cessation of the injections. There was not the slightest palpable enlargement of the uterus or ovaries.

No. 94, a much smaller animal, received 590 rat units, *i.e.*, in proportion to weight, double the dosage of no. 36; the vaginal desquamation rose, remained high (25 to 30 per cent) for seven days after the last injection. There was not the slightest enlargement detectable in the uterus, which remained infantile or about "lead pencil" in size. No bleeding whatever occurred until 15 days after the last injection, when there was sufficient blood lightly to tinge the vaginal lavage. This continued for 4 days. The long latent period in the bleeding response remains to be accounted for.

To the preceding account should be added the results of four laparotomies and two autopsies upon the experimental animals: twice there was no visible change in either ovaries or uterus, twice a slight increase with no bleeding resulting; twice (implants) very considerable Smith-Engle

effect, with bleeding. It should be especially emphasized that bleedings occurred in these animals from small, even infantile uteri—as we have repeatedly seen in spontaneously menstruating females in non-ovulatory cycles.

From the preceding series of experiments the hypophyseal rôle in uterine bleeding is demonstrated in a menstruating animal. But more than that, it is seen that bleeding may be brought on by a much smaller dose of either Amniotin (in animals possessing intact hypophysis) or of anterior lobe (in any form and in any female) than is required to produce visible effects in ovaries or uterus; in fact such effects may be absolutely zero as far as macroscopic changes in these organs are concerned. The threshold for bleeding must, therefore, be many times lower than that for pregravid uterine change or even change accompanying ovulation. There is a profound adaptive significance in this relation, for uterine bleeding fundamentally is an accompaniment of pregnancy, since blood is necessary for the growing young embryo; but "oestrous" changes are incompatible with gestation. Upon these and similar considerations a new theory of menstruation must be built up.

The low threshold for bleeding in the monkey is quite comparable to the low threshold for vaginal cornification as compared with full uterine and copulatory oestrus, which Marrian and Parkes have shown to be as 1:200 in the mouse. It would thus seem possible to produce bleeding in a woman much more readily than stimulating a refractory ovary, and hence a dormant uterus, into action. What benefit a patient would derive from such partial treatment the clinician will have to determine. At any rate the experimental results here presented would seem to enforce a new viewpoint upon the menstruation problem from a therapeutic standpoint.

It appears, further, that bleeding from the uterine mucosa is a phenomenon independent of hyperplasia, swelling, or even congestion of the tissues. The best explanation that occurs to us is that bleeding is due to a hormone elaborated by the anterior lobe separate and distinct from that of growth, follicle stimulating (Smith and Engle), luteinizing (Evans and Simpson), or thyroid-activating (Smith; Crew and Wiesner). We might with reserve speak of a fifth anterior-lobe hormone.

It does not follow, of course, that bleeding is normally an independent phenomenon. We are fully aware of the interactions of all parts of the body and the endocrines in particular. Thus, normally in women and during the breeding season in apes the follicle stimulating and the luteinizing hormones come into play, ovulation occurs, the anterior lobe is stimulated, and bleeding occurs from a "prepared" or pregravid endometrium of the Hitschmann and Adler type. But the stimuli coming from the ovary (or other link in the endocrine chain) may be insufficient to liberate

the first two but quite adequate to liberate the hormone causing bleeding. In the absence of all these hormones—amenorrhea.

SUMMARY

1. Injections of Allen-Doisy hormone (*e.g.*, in the form of Amniotin Squibb) have been known to cause bleeding in female monkeys, whether normal or castrated. It is here shown that this also occurs consistently in baby monkeys 1300 to 1700 grams in weight, about a year old and 2½ to 3 years before the menarche—that is, corresponding to a human child three or four years of age.

2. That this effect is not a direct one is shown by the fact that hypophysectomy abolishes the effect. But bleeding occurs in hypophysectomized as well as normal animals by injection of anterior lobe extracts or by implants of the fresh gland. This is interpreted as a direct effect. A physiological basis of uterine bleeding is, therefore, for the first time demonstrated and the menstruation problem is lifted from the realm of theory and conjecture.

3. It seems reasonable to postulate a separate anterior lobe hormone as the direct cause of bleeding, since it is here demonstrated that the bleeding is independent of the follicle stimulating effect of Smith and Engle and the luteinizing phenomenon of Evans and Simpson and other influences attributed to the gland.

4. With the treatments here outlined bleeding may result from a uterus that shows not the slightest macroscopic change. Hence the threshold for bleeding is far below that causing "oestrous" changes (growth, edema, congestion) in uterus or ovaries. This has adaptive significance, for the prime and original function of the bleeding is that of supplying blood cells (perhaps for iron) directly to the embryo in early pregnancy; "oestrous" changes would be disastrous to the continuance of pregnancy.

5. Since uterine bleeding may be caused so much more readily than hyperplasia of dormant reproductive tract and ovaries, the gynecologist must determine what benefit the patient receives from a mere bleeding without the usual concomitant "oestrous," not to mention pregravid changes.

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Addendum: *Experiment with hypophysectomized monkeys.* Houssay and Magenta and independently Geiling, Campbell and Ishikawa showed that hypophysectomized dogs are hypersensitive to insulin. It was thought advisable to test the reactivity to insulin of the hypophysectomized monkeys used in this work. If they are hypersensitive to insulin then it would be added proof that the hypophysis had been removed, or that the hypophyseal secretion has been seriously impaired.

Four monkeys are used in this experiment; three were operated upon (nos. 20, 36 and 94) and one was a normal control (no. 17). The animals were starved for

Experiment with hypophysectomized monkeys

Blood sugars, mgm. per cent

NUMBER	WEIGHT	DOSE PER KILO	TOTAL DOSE	NORMAL	1 HOUR	2 HOURS	3 HOURS	REMARKS
	kgm.							
20*	4.2	0.5	2.0	70	15			Twitchings, comatose, glucose intravenously and intraperitoneally. Recovery
36*	4.1	0.5	2.0	93	22	33§		Twitching. Very weak. Glucose
94†	2.5	0.5	1.5	116	60	67	79	No reaction
17‡	4.3	2 units	8.6	93	38	53	63	No reaction

* Animals 20 and 36—completely hypophysectomized.
† Animal 94—the gland was not completely removed.
‡ Animal 17—normal control.
§ 1½ hours.

24 hours previous to the test. Normal blood samples were taken from the superficial vein in the hind leg and insulin was injected, immediately after securing the normal specimen, by interchanging syringes without removing the needle. Further blood samples were taken or injections of glucose were made into the same vein.

The operated animals received only one-half unit of insulin per kilo while two units per kilo were given to the normal control. The two animals with the pituitary body most completely removed showed a very marked reaction to the small dose, whereas the normal control had no reaction. The changes in blood sugar are indicated in the chart.

The data indicate clearly that monkeys with the pituitary gland removed are hypersensitive to insulin.

A FURTHER STUDY OF THE HORMONE OF THE ADRENAL CORTEX[1]

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In 1927 we (Hartman, MacArthur and Hartman, 1927) first published evidence of a hormone in the adrenal cortex (see also Hartman, Brownell, Hartman, Dean and MacArthur, 1928; Hartman, Griffith and Hartman, 1928). The average survival period of totally adrenalectomized cats was decidedly increased by the injection of an extract prepared from the cortex. This hormone which is essential to life we called cortin.

We have since been able to prepare a more concentrated extract which will prolong the lives of adrenalectomized animals indefinitely. In this paper we describe the work which led up to this method of preparation and give an account of the use of the more potent extract.

We also report a series of controls because there seems to be a wide difference in the results of various investigators.

Controls. We have studied thirty-five control cats which were treated in exactly the same manner as those used to test cortical preparations, except that they were not all injected. Twenty were not injected or handled. Fifteen were injected with Locke's or isotonic NaCl solution. Those not injected lived from 2 to 35 days with an average of 11.4 days. There was approximately one week intervening between operations in this series. The time of the operation ranged from five to thirteen minutes. This is from the incision of the skin to the sewing of the body wall. The time during which ether was administered ranged from thirteen to thirty-two minutes.

Reduced operation time tends to increase the promptness of recovery but it is doubtful whether it is an important factor in the survival period provided it is not greatly prolonged. Obviously, care must be taken to prevent shock.

Cats injected with NaCl or Locke's solution lived an average of six days or a little more. The frequent handling may have been a factor in shortening the period. In eight animals more than one month intervened between

[1] This was aided by a grant from the National Research Council.

operations, yet the survival period was no longer than in those with but five days intervening.

Preparation of cortin: By precipitation. In preparing cortin one is confronted with the problem of getting rid of the epinephrin, for even with the medulla removed the cortex contains a considerable amount, perhaps one-fifth as much epinephrin as the medulla (Hartman and Hartman, 1923). An active extract nearly free from epinephrin can be obtained by precipitation with NaCl (Hartman, MacArthur and Hartman). So much of the potency is lost, however, by reprecipitation that further concentration of the hormone by this method is impossible.

Heating the extract, from the first NaCl precipitate, to 80°C. for five minutes destroys its potency. Six cats treated with such an extract survived no longer than the controls. It is impossible, therefore, to remove the protein in this manner.

The addition of ethyl alcohol to make a concentration of 50 per cent or ore precipitates the proteins coagulable by heat and nitric acid, but does not destroy the potency. Nine adrenalectomized cats treated with this extract survived an average of 25.4 days, the individual survivals being 6, 9.5, 16, 16, 18, 30, 37, 40 and 57.5 days. Cortin, therefore, is soluble in alcohol. The direct use of alcohol to extract the tissue itself possesses the disadvantage that epinephrin is also dissolved.

By extraction with solvents. We have tried a number of solvents in our attempts to obtain a concentrated preparation of cortin. Low boiling solvents seemed best because high temperatures are not advisable.

Ethyl ether has proven to be the most satisfactory solvent. One or more volumes of ether are added to minced fresh cortex in a flask, the air being replaced by CO_2. This is shaken from time to time, the ether being changed once or more every twenty-four hours until two or three days have elapsed. The portions of ether are combined and filtered. The ether is removed *in vacuo.* The residue is extracted three times with 80 per cent ethyl alcohol warmed to 40°C. The alcoholic solution is cooled to -10°C and filtered cold. The alcohol is removed by vacuum distillation, the temperature never being allowed to go above 45°C. The residue is again taken up with a small volume of 80 per cent alcohol. This is chilled, filtered and distilled to dryness. The residue is extracted with a small volume of ether, the insoluble material being discarded. After removal of the ether the residue is extracted with enough water to make the desired concentration. We have used concentrations containing the material from 10 to 50 grams of fresh cortex per cubic centimeter of extract.

An extract made in this way is nearly free from epinephrin and contains 0.02 mgm. or less of dried substance for each gram of fresh cortex.

It is possible to keep adrenalectomized animals alive and in good condition indefinitely with such an extract. We have five cats maintained by

extract in good condition at 80, 101, 108, 121, and 217 days after removal of the second adrenal (cats ON, LT, RV, RM and NY; see protocols and tables). One cat died at 41 days because the extract was discontinued. A second cat died because the extract was given by mouth. A third cat died from an infection started at the time of the injection of a toxic extract, while a fourth cat died from the strain of a double adrenalectomy at one operation.

We have been able to maintain the growth of young rats after complete adrenalectomy by the injection of cortin. Two adrenalectomized rats brought to maturity in this way are successfully raising litters of young. We are now using the growth of young adrenalectomized rats stimulated by cortical extracts as a means of assaying cortin. Details of the method will be published later.

Weights of treated cats. Treated adrenalectomized cats usually gain in weight if a sufficient amount of cortin is administered. However, if just enough cortin is given to keep the animals in good condition they do not eat so much and gain more slowly. Young cats as would be expected gain more rapidly than older ones. We have tried to give a little above the minimal amount of cortin that would maintain good health except from time to time when testing some variation in the extract. Then the potency might be too low and the weight drop temporarily. The accompanying table gives typical changes in weight.

If more cortin is administered the appetite is greater and the weight increases. If the amount is reduced appetite begins to fail and weight is lost. The last weights shown in table 1 were taken after the amount of extract had been reduced for a few days.

Appetite. The appetite is a fair measure of the condition of the animal although in untreated adrenalectomized animals, occasionally an individual may eat heartily the day before he dies.

We keep a record of the food eaten by all adrenalectomized cats. If one begins to lose his appetite we increase the dosage of cortin. The amount of food eaten is as great or greater than that eaten by normal cats.

Behavior. If one knows the individual cat from having watched him daily he can readily tell when he is not getting enough cortin. When the amount administered is adequate the cats behave normally. They stretch and clean themselves, jump considerable distances to leave or return to their cages, clamor for food, run, play and fight. They are alert and responsive to their surroundings, the younger ones playing with moving objects. Some animals become so aggressive under adequate cortin administration that they struggle strenuously when injected. One animal came in heat and became pregnant (see protocol NY).

Reduce the cortin below the minimum and the same animals no longer object to injection, lose their appetites, become lackadaisical and develop asthenia.

TABLE 1

Weights of treated adrenalectomized cats

First column days after removal of second adrenal, second column weight in kilograms in each instance.

RV ♀ YOUNG		LT ♀ OLD		ON ♂ OLD		RM ♀ PREGNANT	
0	2.61	0	3.25	0	2.95	0	2.45
6	2.52	7	3.05	9	2.86	19*	2.20
30	2.67	23	3.25	17	3.00	37	1.95
44	2.83	76	3.43	55	3.10	57	2.07
83	3.15	80	3.20	59	2.99	96	2.22
87	3.05					100	2.20

* Abortion on 18th day.

TABLE 2

Blood ureas in cortin treated adrenalectomized cats

CAT	DAYS AFTER REMOVAL OF SECOND ADRENAL	TIME	BLOOD UREA, MGM. PER 100 cc.
LT ♀ 3.25 kgm.	2	9:49 a.m. cortin injected	85.3
		1:40 p.m.	53.3
		4.10	43.0
	5	9:40 a.m. cortin injected	49.2
		10:40	39.2
		2:00 p.m.	34.0
		4:00	36.6
		6:00	38.0
		9:00	38.3
	43	9:50 a.m. cortin injected	83.14
		12.25 p.m.	74.0
	71	10:25 a.m. cortin injected	53.83
		1:55 p.m.	65.33
RM ♀ 2.45 kgm.	22	9:28 a.m. cortin injected	74.2
		1:20 p.m.	95.1
		3:55	50.6
	30	9:35 a.m. cortin injected	85.84
		12:17 p.m.	69.27
	40	10:00 a.m. cortin by mouth	43.59
		12:50 p.m.	47.13
		3:30	46.01
	58	10:00 a.m. cortin injected	45.41
		1:45 p.m.	39.19

Blood urea. Blood urea in treated adrenalectomized cats sometimes runs higher than in normals. The essential difference between our findings in the animals treated with concentrated extract and those treated with dilute extract (Hartman, Griffith and Hartman, 1928) is that in spite of the larger food consumption the blood ureas are no higher in the former group of animals. Allowance must be made for considerable variation in the blood urea of normal cats. Typical results are shown in table 2. In the morning twelve to fifteen hours after the last injection the urea is sometimes high. If so, the injection of cortin usually lowers it. Rarely the injection of cortical extract raises the blood urea temporarily (see RM on the 22nd day). When the blood urea is already low, cortin has little effect.

Cortin administered by mouth had no effect on urea (see RM on the 40th day). However the urea was so low that little significance could be placed in this one animal. Cat RV with a blood urea of 65.85 mgm. at 9:55 a.m. showed no change when cortin was given by mouth, the urea being 65.38 at 12:45 p.m. and 65.11 at 3:21 p.m.

The higher urea in the morning does not seem to be accounted for in most cases by food because feeding took place 15 to 20 hours before. Indeed LT was not fed for 60 hours before the value 85.3 mgm. was obtained on the 2nd day. This particular value may have been a post-operative effect. However, it is interesting to note that cortin brought it down.

A low blood urea may accompany an excellent general condition of the animal. For example, the value fluctuated between 66 and 105 mgm. during the first four months following complete adrenalectomy in cat NY, while in the sixth month when the cat was at her best the urea fell to 30–35 mgm. As pregnancy progressed and the extract was discontinued for a time the urea again rose to 53–57 mgm. This is not always so. In cat RV whose condition has always been excellent the urea has rarely gone below 50, often being 70 to 90. Cat ON had a blood urea of 39 to 45 before adrenalectomy and 55 to 84 afterward, although in excellent condition.

Recovery from injury and resistance to infection. It has been our experience as well as that of others that completely adrenalectomized cats recover poorly from operative injuries or from infections which are easily contracted by them. Injection with cortin adequate in quantity and frequency changes the whole picture to that of the normal animal.

Wounds heal promptly. There were two swellings under the skin in cat LT thirteen days after the second adrenal removal. Two incisions were made for exploration. Clotted blood was found. The wounds were left open, healing rapidly. Healing seems to occur as early after the second operation as in normal cats with adrenals.

We have lost but one animal from an infection which was not drained. This probably would have killed a normal animal.

Some of our treated animals have had slight infections of the respiratory tract but they quickly recovered. This is strikingly different from untreated adrenalectomized cats.

Infections, repair of injuries and conditions of stress demand more cortin. If it is not available, signs of insufficiency quickly develop. This is particularly well demonstrated in cats that aborted (NY and RM) and in cat OZ that had a skin infection from a severe iodine burn.

It has, heretofore, never been possible to remove both adrenals in the cat at one operation and maintain life for many days afterward. If a sufficient amount of cortin is available for frequent injection this is possible. Cat OZ illustrates. He died because administration by mouth was attempted. Otherwise there was every indication that life could have been maintained indefinitely as he seemed perfectly normal at the time the method of administration was changed.

Recovery from acute adrenal insufficiency. Four adrenalectomized cats have been brought to a condition of acute insufficiency by the administration of inadequate amounts of cortin or the absence of cortin entirely. In one, recovery failed because the extract used was impotent. This was demonstrated by the failure of appetite in other animals in which it was used. The other three were revived by frequent injections. (See cats OZ, NY and RM.) Two became prostrate and dyspneic, one showing muscular twitching. The extremities were cold and all indications pointed to death in a few hours, yet cortical extract revived them and brought them back to health. At such crises the injections must be prompt, frequent and adequate in potency.

In this late stage which has been frequently described in the literature, the animal not only gives a picture of distress because of his rapid breathing but before the stage of prostration he may move tottering from one position to another in the cage or room, but finds no place satisfactory. He cries out and seems uncomfortable. He hunts a warm place if he can find one, although as a rule he does not shiver at this time.

The injection of cortin causes first a disappearance of restlessness, twitching and dyspnea and then a return of physical power. Appetite is regained somewhat later.

When adrenal insufficiency is slow to develop recovery seems to be slower. In contrast the recovery is rapid if the insufficiency came quickly (cats OZ and RM).

In cat RM it was remarkable to see an animal apparently moribund sit up a little more than one hour after the injection and still more striking to see it eat fairly well in less than two hours.

At a certain stage in recovery some animals shiver for a few hours in a room temperature of 25°C. However, this soon disappears.

Relation of cortin to the skin and hair. Adrenalectomized cats that sur-

vive many days without treatment frequently show alterations in the skin. It becomes dirty grey in appearance and the hair loosens and is shed profusely. This condition is also present in many animals whose lives are prolonged by the injection of cortin (Hartman, Griffith and Hartman, 1928). In the present study where larger amounts of cortin were injected a similar condition was often noted during the early period after the operation. After a few days or weeks the condition entirely cleared up, the skin becoming fresh and pink in appearance when shaven. New hair grew, the coat becoming sleek and fresh.

Discussion. It is now well established that the adrenal cortex produces a hormone which is essential to life. Our previously published work (reference already cited), together with the present work and that of Swingle and Pfiffner place this beyond the shadow of a doubt.

This substance, which we have chosen to call cortin, profoundly affects the various systems of the body. The circulatory, muscular, nervous, gastro-intestinal and excretory systems become involved. Which are primary and which are secondary effects has not been determined.

Evidence that the circulatory system becomes involved is meager in animals. In the late stages the blood solids increase, blood flow through the smaller vessels, at least in the periphery, seems to decrease and coagulation occurs more readily. In Addison's disease the blood pressure may become low. The injection of cortin causes decided improvement in the circulation. A case of Addison's disease (unpublished work of Hartman, Aaron and Culp) that had a systolic blood pressure of 50 mm. when brought under observation responded to injections of cortin by a rise of 74 mm. in twenty-four hours. In four days the pressure had reached 94 mm. Before treatment the pulse was very feeble with a rate of 120 or more per minute. By the seventh day after treatment started the pulse was strong with a rate of 70 per minute. When the amount of cortin was reduced the blood pressure again fell.

Asthenia which is an outstanding symptom of adrenal insufficiency is caused by a lack of cortin, because when an adequate amount is supplied the asthenia disappears. Little is known regarding the influence of cortin on the nervous system. In adrenal insufficiency restlessness and increased irritability may develop in both animals and man. A lack of interest in the surroundings and a failure to respond to external stimulation are also signs of insufficiency. Cortin abolishes these symptoms.

The relation of cortin to heat production and regulation requires investigation. In adrenal insufficiency, heat production falls, the extremities become cold and the rectal temperature falls. Upon recovery produced by cortin injections, an animal may shiver for several hours or longer in a warm atmosphere.

The loss of appetite is probably due to the gastro-intestinal disturbances. Loss of weight must be due to both.

Cortin seems to stimulate a kidney with lowered function at least that present in adrenal insufficiency. This is indicated by the fall in blood urea when high. In the case of Addison's disease mentioned above the blood urea was lowered from 120 mgm. per 100 cc. to 22 to 60 mgm. This is not entirely a renal phenomenon, however, because the fluid intake is reduced, the circulation is sluggish and the blood itself changes for it clots more readily. Adrenalectomized cats which are supplied inadequately with cortin drink less water and secrete little urine. If given ample cortin they drink freely and urinate copiously.

Pregnancy seems to increase the demand for cortin. Two pregnant adrenalectomized cats were not given the additional cortin needed. Both aborted. Two adrenalectomized rats were raised to maturity and became pregnant. During pregnancy and lactation they required more cortin to maintain weight and health. Reduction in the cortin caused them to lose weight and die.

Growing animals require proportionally more cortin than adults. Young adrenalectomized rats have been raised to maturity by the injection of cortin. Some require it only once daily while others need more frequent injections. If deprived of cortin many die within a few hours or days. Adults similarly treated survive somewhat longer.

Cortin is required in varying degree. During quiet life the needs are minimal, but increased activity of the tissues demands more of the hormone. This is true in anesthesia, trauma, repair, infections and muscular activity.

The results of cortin injection begin to be evident in the course of a few hours. Its action is not as prompt as that of some hormones. The quickness of the response seems to depend partly upon the acuteness of the insufficiency. If the insufficiency has developed slowly it seems to require longer to produce a reaction with cortin. On the other hand when the insufficiency is acute a response may be noted inside of an hour or so.

Cortin administered by mouth appears to give little or no benefit. It is best given subcutaneously except in circulatory failure where absorption is very much reduced. Then intravenous injection should be employed.

The amount of cortin needed daily to maintain adrenalectomized cats is that obtained from 10 to 20 grams of cortex per kilo of body weight.

We wish to express our indebtedness to the Hygrade Packing Co., Buffalo; Dold Packing Co., Buffalo; Armour Packing Co., Chicago; and Wilson Packing Co., Chicago for the careful preparation of adrenal glands for this work.

SUMMARY

Cats whose adrenals are removed in two stages survive from 6 to 11 days when untreated. Those injected with a small quantity of isotonic salt

solution survived the shorter period. Increase in the interval between the operations, above a few days, does not lengthen the survival period. An increase in the operation time when not too great does not decrease the survival period. Adrenalectomized animals show considerable loss in weight if they survive many days.

Cortin, the hormone of the adrenal cortex which is essential to life, is carried down by the precipitated material formed when NaCl is added to the point of saturation or below. Adrenalectomized cats treated with an extract made from such a precipitate survived an average of 26.4 days.

Heating the NaCl precipitate extract to 80°C. for five minutes destroys its potency. Precipitation of the protein in the NaCl precipitate extract by ethyl alcohol does not diminish the potency.

A concentrated preparation of cortin can be made by extracting the cortex with ethyl ether which dissolves very little epinephrin. The ether is removed by distillation, the residue being extracted with warm 80 per cent ethyl alcohol. Much inert material is removed by chilling and filtering. After removing the alcohol, the residue is taken up in water. The aqueous solution contains the cortin.

This extract acts as a substitute for the adrenal cortex. Adrenalectomized cats are being kept alive in good condition indefinitely by its use. The growth of young rats whose adrenals have been removed has been maintained until the adult stage is reached by injecting the extract. Such rats behave normally in every way, even to raising litters.

Cortin treated adrenalectomized cats eat well, gain in weight, play, fight and act like normals. Cortin lowers the blood urea when high. It increases the resistance to infection and to cold. It speeds the repair of injury and enables the animal to withstand operative or other trauma. By its use it is possible to remove both adrenals at one operation and maintain life indefinitely.

Animals allowed to pass into the last stages of adrenal insufficiency which just precede death have been revived by cortin.

A case of Addison's disease with a systolic blood pressure of 50 mm. and a pulse of 120 per minute together with other characteristic symptoms has been revived by the use of cortin.

Cortin is apparently not effective by mouth. When given subcutaneously one can begin to detect its effects in a few hours.

From protocol of cat NY ♀, 2.17 kgm. Adrenals removed in two stages. Animal injected with a very small amount of cortin (NaCl precipitate extract) twice daily. Ate small amount of food (50 to 80 grams daily). Weight dropped as low as 1.73 kgm. after one hundred days. Twice the extract was discontinued for two and three days. Appetite failed so that injections were renewed.

At 105 days the use of a more concentrated extract (ethyl ether extract) was begun. The cat responded within 24 hours by taking more food than usual. She

soon was eating 100 to 150 grams daily. She became much more active and gained in weight. In six days she was in excellent condition. At 123 days, weight 1.93 kgm. At 136 days, anesthetized cat with ether. Through an opening in the mid-line abdomen explored thoroughly for cortical tissue. None was found. Anesthesia 36 minutes. Operation 16 minutes. Recovery from operation excellent. In four days cat eating as well as ever.

At 152 days weight 2.18 kgm. Cat being in heat was placed with a male.

At 157 days extract discontinued. Her appetite failed somewhat and she became weak. Twenty-six days from the time of stopping the extract she refused food. Her weight was 2.33 kgm. In spite of this increase she was very thin, the increase having been caused by pregnancy. Extract was again administered twice daily in the usual dosage. She steadily grew worse and nine days later aborted five fetuses 7 cm. long. The amount of extract as well as the frequency of the injections was increased. She showed definite improvement and three days after the abortion began to eat. In a few days her appetite returned and she regained her former condition. It has now been 217 days since removal of the second adrenal.

From protocol of cat RM ♀, 2.45 kgm. Adrenals removed in two stages. Animal appeared fairly well until 18 days after removal of the second adrenal when an abortion of very small fetuses occurred. Bleeding continued for eight days. Hair loose, easily pulled out. Appears lackadaisical. Extract (ethyl ether) not given for twenty-two hours because animal then appeared in good condition. At end of that time cat could barely stand when stimulated, otherwise sat with head drooping on the floor. Injected at this time. Three hours later, cat sat up, unquestionably better. Five hours later, much better. Stands up and cries for food. Injected three times on this day, 12 hours elapsing between the last injection and the observation next morning.

At 9 a.m. the next day extract, which later proved to be poor, was injected. By 11:50 a.m. cat was lying on side, prostrated, muscles twitching as other untreated adrenalectomized cats have done just before death. At 12:05 p.m. a different extract injected. At 1:00 cried out and moved front legs. At 1:20 p.m. sat up. At 3 p.m. ate 40 grams of mackerel. At 3:55 p.m. shivering although the room was warm. At 8 p.m. completely recovered, walking about.

On the following day (34 days after adrenal removal) animal appeared alert and in excellent condition when injected with cortical extract (5 a.m.). But at 1:55 p.m. cat again so weak that she fell over when attempting to stand. A larger amount of the same extract which proved later to be impotent was injected at this time with no result. Animal became steadily worse until at 2:50 p.m. she was prostrated and showed dyspnea and marked convulsive twitches in different parts of the body. A small amount of a different extract was injected intraperitoneally at this time. Sixty minutes later the twitching and dyspnea had ceased. Seventy minutes later she was sitting up. Eighty-five minutes later she was shivering. A heater was placed near. One hundred minutes later she ate 50 grams of fish. Two more injections of the potent extract were made on this day. The animal cried out for more food and was given 35 grams. On the following days the cat appeared in good condition except that for five days she would frequently shiver at a room temperature of 25°C. She ate well and showed vigor in her activitites. About fifty per cent more extract than was used before the abortion was given daily (in two injections). Fifteen days later the amount of extract administered daily was reduced one-half. After twelve days of this reduction her appetite was not quite as good as usual, so that the extract was increased.

Seventy days after the adrenals were removed the extract was given by mouth

in a small quantity of milk. After five days of this method of treatment the appetite fell so that the food intake was reduced nearly one-half. Injections were resumed and the appetite promptly returned. It has been 121 days since the removal of the second adrenal.

From the protocol of cat OZ ♂ 2.60 kgm. Both adrenals were removed at one operation by the lumbar path on each side. Cortical extract (ethyl ether) was injected immediately and twice daily. Made excellent recovery.

4th day. Not so well. Bloody diarrhea.

5th day. Vomited. Extract given three times instead of twice.

6th day. Better—feces hard.

7th day. Refused food at usual time. Extract for last two injections not potent as indicated by other animals. OL died from being administered the same. Two injections of large amounts (4 and 5 cc.) of extract 6 hours apart made cat regain his strength and he ate 140 grams of fish.

8th day. Stitch abscesses discovered on one side. On days when cat appeared to need it, three injections were made instead of two.

11th day. The ordeal of dressing opened stitches on the right side and large sore on left side from an iodine burn was so great that cat became prostrate, dyspnea set in, extremities became cold and the animal appeared about to die. Four injections of cortical extract representing material from 180 grams of cortex altogether were made in the course of fourteen hours. In 5.5 hours after prostration the cat sat up and ate a little meat and broth. Bloody diarrhea.

12th day. Three injections, totalling material from 240 grams of cortex, were made. Cat improving.

14th day. Much better but bloody diarrhea. Three injections. Cat steadily gaining so that by the 21st day wounds were nearly healed and condition was excellent.

24th day. Extract given by mouth in a small quantity of milk. This method of administration was continued for five days when both milk and most of fish was refused.

29th day. Injections resumed but the animal was not carefully watched. He died in less than 10 hours after injections were resumed. Right heart partially filled with fibrin. Tapeworms in small intestine.

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THE NERVOUS CONTROL OF RESPIRATION IN KITTENS

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Certain observations made on very young animals in an extended study of the neuro-muscular mechanism of respiration have led us to suspect that there are certain differences in the action of the respiratory mechanism of the young as compared with adult animals. One of us has therefore made a systematic survey of the nervous mechanism for the control of respiration in kittens from birth to sixty-three days old. We selected kittens for this study because our available data on cats afford a basis of comparison between the older and the younger forms.

Experimental procedures. Ether was given by tracheal cannula. The anesthesia was kept fairly light and in many cases could be intermitted for long periods. Small stethographs were placed around the thorax and abdomen at the level of the diaphragm and connected with recording tambours to register costal and diaphragmatic respiration. In a few cases, respiration was registered from the tracheal cannula connected directly with a recording tambour. After taking a control record of respiration, the analysis of the nervous mechanism was begun by a study of the effects of section of the elements, afferent, central and efferent, which our previous experience indicated belonged to this nervous mechanism.

Division of the vagi. Silk ligatures were placed around each vagus in the neck. One nerve was then divided and a record of thoracic and diaphragmatic movements was taken. After an interval of about a minute, the second nerve was divided without interrupting the record. While section of one vagus does not cause much change in the form of the respiratory movements, it appears that following double vagotomy, there is not only the slowing of rate observed in the adult, but there is sometimes a diminution in the amplitude of the diaphragmatic respiratory movements which is not observed in older animals. Often, also, the diaphragmatic movements take on the sobbing form usually seen in the adult cat only after section of the midbrain (Coombs, 1930). In short, double vagotomy

[1] The experimental portion of this paper was prepared by Doctor Coombs and the discussion was prepared by Doctor Pike. The cost of animals for the study was met by a grant to Doctor Coombs from the American Association for the Advancement of Science.

in the kitten appears to affect diaphragmatic respiration more than in the adult. Moreover, cats with both vagi divided can live indefinitely, but this appears not to be the case with young kittens or puppies. They usually survive double vagotomy for only a few hours or even less. Just as in the case of full-grown cats, there are "vagus" kittens in which the functional activity of the vagus appears to assume a rôle of greater importance than in other kittens, and the proportion of these "vagus" kittens seems to be higher than in the case of full-grown cats. In such animals, following double vagotomy, gasping and dyspnea followed by death supervene earlier than in others. Even in kittens forty to fifty days old, double vagotomy causes death in a few hours. Several such animals had the vagi cut aseptically in the hope that they might survive. None did, however.

Section of the dorsal spinal nerve roots. Laminectomy in the thoracic or cervical region, or both, was done; the laminae being broken off bit by bit with a pair of forceps as gently as possible, since rongeurs or bone forceps are too heavy to use in work on new-born kittens. Great care was taken to avoid hemorrhage. Judging by the form of the respiratory movements after completion of the laminectomy—similar to the control records—respiration was not appreciably affected by the preliminary operation. When clearly exposed, the dorsal roots were cut with a pair of fine scissors, and further tracings of the respiratory movements were taken.

When the dorsal roots of the spinal nerves are divided in the thoracic region, costal respiration in kittens from birth to ten days old almost ceases; it is diminished in older kittens, but to a less extent than in the very young animals. But, since normal costal respiratory movements are relatively slighter in kittens than in full-grown cats, the diminution in them which comes after section of the dorsal spinal nerve roots may be of less significance than in adult animals. Following such an operation, failure of costal movements is adequately compensated for from birth onward by an increase in the depth of the movements of the diaphragm.

When the dorsal roots of the cervical nerves are sectioned, the thoracic nerves being intact, the movements of the diaphragm are much cut down and the respiratory rate is slowed, at no matter what age (Coombs, 1929). In adult cats, compensation for such a section is effected by means of deeper costal respiration, but this is not the case in young kittens, even though the thoracic spinal nerve roots are all intact. It has been found that there is very little thoracic compensation for diminution or failure of diaphragmatic respiratory movements under twenty to twenty-five days of age.

When the dorsal roots of both thoracic and cervical nerves are divided, kittens live only one or two hours and respiration is dyspneic. This is particularly the case in kittens under two weeks of age, in which no compensation takes place.

Section of the midbrain. After temporary occlusion of the carotids with serrefines to decrease hemorrhage, the sutures of the parietal bone were cut in the midline—the skull being as yet not very much ossified—and followed around laterally just over the tentorium. Guided by the tentorium, a thin-bladed knife was pushed down vertically just behind it. In this way, either unilateral or bilateral section of the midbrain could be done without much hemorrhage. It was found that section of one side at a time was less likely to cause a disturbance of the respiratory movements than if total transection were done all at once. Following the death of the animal, the operation was always checked by autopsy to verify the location of the section. Section of the posterior corpora quadrigemina in young animals slows respiration somewhat, but it does not give rise to the uncontrolled movements of the diaphragm which are frequently observed in old cats after this procedure. Moreover, costal respiration remains unaffected in those kittens from one to ten days old in which the midbrain has been divided. A tracing taken from the trachea of an eight day old kitten shows the regularity of the respiration following transection of the midbrain (fig. 1). The younger the animal, also, the longer appears to be the period of survival after the operation. The longest survival period noted was that of a two-day old kitten which lived twenty-four hours after complete section of the midbrain, during which time respiration remained unaltered. It is probable that death, when it occurred, was due to other causes than the primary action upon respiration. By the time that costal respiration begins to appear, the survival period following section of the midbrain is much cut down.

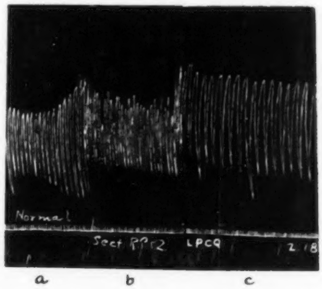

Fig. 1. *a* shows normal respiration, recorded from the trachea; *b* shows respiration after section of the right posterior corpus quadrigeminum; *c* shows respiration after section of the left posterior corpus quadrigeminum, or total transection of the midbrain. Note the regularity of the respiratory movements after the midbrain has been divided. This animal was eight days old.

Section of the phrenics. Silk ligatures were placed under the phrenics in the neck, and the nerves were then removed, care being taken to get all three branches on each side, so as to deprive the diaphragm completely of phrenic innervation. The paralysis of the diaphragm thus induced is attended by more severe results in kittens than in full grown cats. The fact that the costal mechanism is unable to make adequate compensation is emphasized very strongly by this operation. It was shown earlier in the paper that when the dorsal roots of the phrenics are divided in the

cervical region, there is no costal compensation for the reduced diaphragmatic respiration. When the entire phrenic is divided, this lack of ability to compensate becomes even more apparent, as respiration fades out after a short time. As the animals grow older, there is a greater degree of compensation which begins to be apparent at about three weeks, and in the 60 to 65 day old kitten, the reaction is that of the adult cat—that is, the thoracic movements have become adequate to maintain respiration indefinitely.

The following table indicates the relative severity of the various single lesions, varying with the age of the animal.

TABLE 1

The effect of single lesions, as related to the age of the animal

LESION	BIRTH-10 DAYS	11-21 DAYS	22-48 DAYS	49-64 DAYS
Division of vagi	Slows respiration. Cuts down activity of diaphragm. Early death	Slows respiration. Cuts down activity of diaphragm. Sobbing movements	Slows respiration. Sobbing type movements of diaphragm	Slows respiration
Division of dorsal thoracic nerve roots	Stops costal respiration	Cuts down costal respiration	Cuts down costal respiration	Cuts down costal respiration
Division of dorsal cervical nerve roots	Cuts down movements of diaphragm. Slows respiration	Same	Same	Same
Excision of phrenics	Stops diaphragmatic respiration. Early death	Same	Stops diaphragm movements. Some dyspnea	Same
Division of posterior corpora quadrigemina	No effect	May cause slightly earlier death	Earlier death. Cuts down thoracic respiration	Earlier death. Cuts down thoracic respiration. Sometimes causes sobbing movements of diaphram

Combined lesions: Division of vagi and midbrain. The effect of division of both vagi and posterior corpora quadrigemina in very young animals (under eight days) appears to be no more severe than division of the vagi alone. Kittens from one to seven days old survived the double operation from two to ten hours. The protocol of such an experiment follows.

December 16, 1929. Kitten, 1 day old. Light ether, tracheotomy

2:40 p.m. Control tracing of respiration
2:45 Both vagi divided. Respiration slowed
2:50 Section behind posterior corpora quadrigemina
3:04 Respiratory record taken
3:25 Respiratory record taken

3:35	Respiratory record taken
3:45	Respiratory record taken
	No dyspnea. Some costal, as well as diaphragmatic respiration. Costal respiratory movements observed
5:00	Respiratory record taken. Costal respiration good
	The animal was then surrounded by warm cotton and placed where it could be observed every few minutes throughout the evening and night
8:00	Respiration regular
10:00	Respiration slower, considerable respiratory effort of the thorax
11:00	Same as the preceding hour
1:00 a.m.	Terminal gasps after a period of dyspnea

Autopsy showed complete section behind the posterior corpora quadrigemina.

It may be noted from the above protocol that costal respiration was unaffected by section of the midbrain, whereas, in older cats, it would be very much cut down. The effect of the combined lesions in this case was obviously no greater than the single operation of double vagotomy.

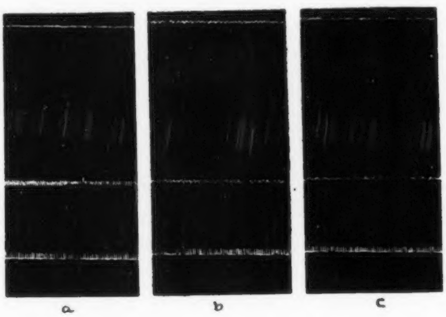

Fig. 2. *a* shows control thoracic (upper tracing) and abdominal (lower tracing) respiration in a 14-day old kitten; *b* shows respiration following cervical laminectomy and section of the cervical dorsal roots. Note the slowing of the respiratory rate. *c* shows respiration following section of the midbrain behind the posterior corpora quadrigemina.

Division of dorsal thoracic nerve roots and midbrain. When division of the dorsal spinal nerve roots in the thoracic region is followed by division of the posterior corpora quadrigemina, the degree of change brought about in the character of the respiratory movements depends to a considerable extent on the age of the kitten. In animals under ten days old, respiratory movements persist for some hours following these two combined lesions and are chiefly diaphragmatic, as in the very young normal kitten. In older kittens, in which section of the midbrain is attended by more marked effects upon the respiratory movements, the survival period is much cut down.

Division of dorsal cervical nerve roots and midbrain. When the dorsal roots of the spinal nerves have been divided in the cervical region, and the posterior corpora quadrigemina sectioned, the age of the animal is again an important factor in determining the effect of the lesions. In the kitten under ten days old, the chief effect of this double operation is the cutting down of the diaphragmatic movements, which are not compensated for by any increase in thoracic respiration. Figure 2 shows this condition in a fourteen day old animal. The protocol of the experiment from which this tracing was taken follows.

February 13, 1930. Kitten, 14 days old. Ether, tracheotomy

11:15 Control record of respiration

11:30 Laminectomy in cervical region and section of cervical dorsal roots
Record of respiration taken

12:05 Section behind posterior corpora quadrigemina; artificial respiration until spontaneous respiration returned at 12.10

12:10 Record of respiration taken

12:25 Record of respiration taken. Note—no compensation by intercostals when diaphragm is not active

12:45 Respiration fades out

Autopsy. Section of the posterior corpora quadrigemina complete except for a small median portion of the tegmentum.

By the time that thoracic compensation for the diminished movements of the diaphragm after section of the dorsal roots of the phrenics occurs, section of the midbrain, which has hitherto been without any marked effect upon the respiratory movements, decreases or eliminates such compensation.

Division of dorsal thoracic nerve roots and vagi. Division of the dorsal thoracic nerve roots followed by division of both vagi, affects the respiratory movements severely. This is to be expected, since division of the vagi alone, without additional lesions, brings about an early failure of respiration. If the following protocol of such an operation be compared with that of division of vagi and midbrain (December 16th), it may be seen that the very young animal cannot withstand division of the dorsal thoracic roots and vagi quite as well as division of the dorsal thoracic nerve roots and the corpora quadrigemina.

December 18, 1929. Kitten, 3 days old. Light ether, tracheotomy

12:30 Control tracing of respiration

12:40 Laminectomy in thoracic region (thoracic respiration disappears)

1:05 Dorsal roots of spinal nerves cut in thoracic (respiration completely diaphragmatic)

1:50 Vagi divided. Respiration becomes labored

2:30 Respiratory record taken

3:05 Respiratory record taken

3:50 Respiratory record taken. Respiration dyspneic

4:30 Respiratory record taken. Terminal gasps

Division of dorsal thoracic nerve roots and phrenics. When, in addition to section of the dorsal roots of the thoracic nerves, the phrenics are excised, there is an early failure of respiration. It has been shown for adult cats (Pike and Coombs, 1922) that if the dorsal roots are divided in the thoracic region, and costal respiration thereby cut down, thoracic respiratory movements will return with greater amplitude than in the control period, if the phrenics are divided. But this compensation does not occur at all in kittens under three weeks of age, and begins to show only slightly at that time. Instead, after a few weak gasps, respiration fades out.

Division of vagi and phrenics. Division of both vagi and phrenics in kittens is attended by early failure of respiration, since in this case, the main afferent and efferent respiratory pathways are cut off. In animals under ten days old, owing to lack of thoracic compensation, death occurs

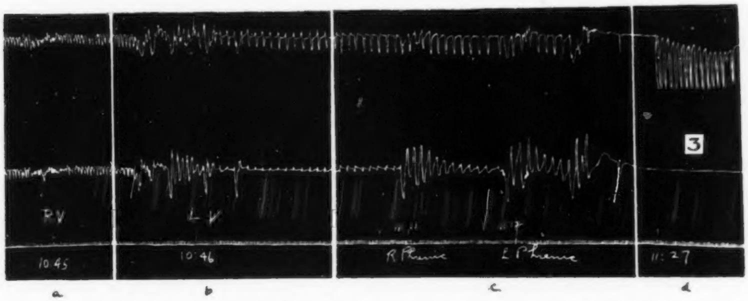

Fig. 3. *a* shows the effect of division of the right vagus in a kitten 26 days old; *b* shows the effect of division of the left vagus; *c* shows the effect of section of the phrenics. Note the costal compensation which has been established in *d*.

usually within an hour. In animals over three weeks old, when some degree of thoracic regulation has set in, respiration fails after a slightly longer interval, as the following protocol shows.

April 21, 1930. Kitten, 26 days old. Light ether, tracheotomy

10:40	Control tracing of respiration
10:45	Right vagus cut, continuous respiratory record taken
10:46	Left vagus cut, continuous respiratory record taken
11:04	Respiratory record taken
11:11	Right phrenic excised, respiratory record taken
11:12	Left phrenic excised, respiratory record taken
11:13	Respiration fails and artificial respiration is given for 3 minutes No ether has been given since 10:45
11:16	Respiration has returned. Artificial respiration stopped. Record taken
11:40	Respiratory record taken
12:15	Terminal gasps

It may be seen from the respiratory tracings of this experiment (fig. 3) that the respiratory mechanism has evolved at this stage beyond the earlier type and that compensation for failure of diaphragmatic movements is being made by the intercostals. On the other hand, it can be seen that the influence of the vagus upon phrenic activity still persists, since section of the vagi not only slows respiration, but cuts down the amplitude of the movements of the diaphragm, which acquire the slightly sobbing character typical of interruption of some part of the proprioceptive pathway (Coombs, 1930).

Division of phrenics and posterior corpora quadrigemina. When phrenics and posterior corpora quadrigemina are both sectioned, the effects of the double lesion are at all times severe, but from different causes. In very young animals, where section of the midbrain is practically without effect, excision of the phrenics alone, causes failure of respiration in a short time, and in older animals, where thoracic compensation has been established, section of the now functionally active midbrain at the level of the posterior corpora quadrigemina interrupts this newly opened pathway at a different level, so that there is equally early failure of the respiratory movements. The following protocol is typical of such an experiment at the period when the thoracic mechanism is just beginning to be active.

April 16, 1930. Kitten, 21 days old. Light ether, tracheotomy

11:18 Carotids temporarily ligated
11:20 Section of right inferior colliculus
11:22 Carotids released, tracing taken
11:26 Complete section of posterior corpora quadrigemina
Respiratory record taken
11:38 Respiratory record taken, respiration is dyspneic, diaphragmatic
12:07 Respiratory record taken
12:30 Respiratory record taken, respiration mostly diaphragmatic
12:40 Right phrenic excised, respiration becomes more costal
12:43 Left phrenic excised, respiration is strongly costal, no active movements of of diaphragm
12:55 Respiratory record taken
1:25 Respiratory record taken
1:53 Respiratory record taken
2:10 Terminal gasps

Autopsy showed complete section just behind posterior corpora quadrigemina. No clots.

It may be noted from the tracing of this experiment (fig. 4) that after section behind the midbrain, respiration regains a regular rhythm. But when the phrenics are removed, respiration becomes irregular and acquires a gasping character as it fades out. This is also the case in adult animals.

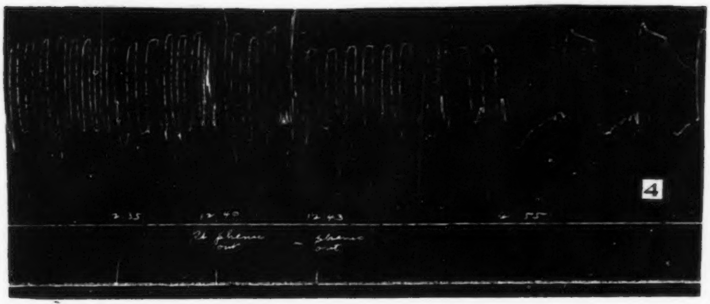

Fig. 4. Shows respiration as recorded by tracheal cannula following complete section of the midbrain at the level of the posterior corpora quadrigemina in the kitten 21 days old, followed by removal of both phrenic nerves. Note the immediate gasping of the respiration.

TABLE 2

Effects of combined lesions on kittens at different ages

LESIONS	BIRTH–10 DAYS	11–21 DAYS	22–48 DAYS	49–63 DAYS
Division of vagi and corp. quad.	Same as section vagi alone. Respiration fails after some hours	Slightly more severe. Respiration fails sooner	Severe effect. Early failure of respiration	Effect same as in adult. Death in 2 to 4 hours
Section dorsal spinal nerve roots and corp. quad.	Effect not marked. No costal respiration	Same as either lesion alone. No costal respiration	Slightly more than section of corp. quad. alone. No costal respiration	No costal respiration. Diaphragmatic movements may become sobbing
Section dorsal spinal nerve roots and vagi	Early respiratory failure. No costal movements	Early respiratory failure. No costal movements	Respiratory failure. No costal movements	Respiratory failure. No costal movements
Section of vagi and phrenics	Early respiratory failure. No thoracic compensation	Early respiratory failure	Later respiratory failure. Some attempts at thoracic compensation	Thoracic compensation established. Animal may survive several hours
Section of corp. quad. and phrenics	Same as section of phrenics alone. Early respiratory failure. No thoracic compensation	Early respiratory failure	Early respiratory failure. Thoracic compensation abolished by lesion	Early respiratory failure
Division of dorsal thoracic roots and phrenics	Early respiratory failure. No thoracic compensation	Early respiratory failure	Some thoracic compensation	Thoracic compensation well established

Table 2 illustrates the effects of combined lesions of the respiratory system, as related to the age of the animal.

Discussion. The differences in respiratory behavior of very young kittens as compared with older animals bring out certain trends in the development of the nervous mechanism of respiration.

While the phrenic mechanism is at all times able to compensate for failure of thoracic respiration—at best poorly developed in animals under twenty-one to twenty-five days old—below this age it appears to be impossible for the costal mechanism to effect a like compensation for failure of diaphragmatic respiration. Moreover, in animals from one to seven days old, the lack of immediate effect upon respiration of division of the midbrain at the level of the posterior corpora quadrigemina, in contradistinction to its marked effect in older animals, shows that as yet that part of the proprioceptive pathway concerned in the regulation of respiration, which traverses the midbrain at that level, has not become functionally active. This is shown in another way by the contrast in the activity of the thorax in adult cats as compared with young kittens, after section of the dorsal roots in the thoracic region, following section of the phrenics. In full grown animals there is a diminution of costal respiration after section of the dorsal thoracic nerve roots. But if, following this, the phrenics are excised, there is a return of costal respiration to a greater depth than before. This compensation does not occur in the kitten under about twenty-one days of age.

The vagus control over the movements of the diaphragm appears to be acquired earlier ontogenetically than regulation by any other portion of the afferent system, and its section is followed by relatively more severe effects in the very young animal, than in the animal in which the nervous system has undergone complete development. This is only to be expected if the vagus is the main source of afferent regulation. Double vagotomy brings about the sobbing type of respiration in the kitten, and also frequently cuts down the magnitude of the movements of the diaphragm, neither of which result from the same operation in the adult cat, in which there are additional afferent impulses coming in from other sources. It is not until the corpora quadrigemina are divided in addition to vagotomy that the diaphragmatic sobbing type of respiration is shown in the full grown animal.

From the evidence at hand, it would appear that at birth the respiratory mechanism has a relatively simple pathway in which the vagi and phrenics are the chief afferent and efferent nerves and the respiratory center in the medulla is relatively unaffected by impulses from nuclei higher up in the brain stem. But as thoracic respiration becomes of greater functional importance, the mechanism of the nervous control of the intercostals superimposes additional regulatory factors and the development of the higher levels of the nervous system multiplies the possibility of central connections above the medulla.

The precise location of the central station or stations at the level of the posterior corpora quadrigemina which are in functional relationship with the afferent pathways from the intercostals has not as yet been definitely settled. It has been shown that such control is ipsilateral (Coombs, 1929). It has been suggested by Allen (1927) that the reticulo-spinal tracts taking origin from the reticular nuclei of the upper medulla, pons and isthmus are respiratory in function, and of these he believes that the lateral reticulo-spinal fibers (both crossed and direct) are the chief ones concerned in the direct conduction of respiratory impulses from cells higher than the medulla to the ventral horn cells of the spinal cord. Since efferent impulses do not arise spontaneously, there are certain possibilities to consider as to the manner in which they are elicited. If these tracts convey efferent respiratory impulses from centers higher than the medulla to the ventral horn cells, their cells of origin must have received impulses by means *1*, of changes in the concentration of the blood gases. They must, therefore, be sensitive to such changes in the same way as are the respiratory cells in the medulla; or *2*, of impulses from the respiratory center itself. These would have to travel on short, ascending pathways and descend again by the route indicated by Allen; or *3*, of afferent fibers from various sources concerned in the regulation of respiration, particularly from the intercostals, which terminate in the central nuclei of the superior and inferior colliculi from which descending fibers carry impulses which are summed up with the blood gas stimuli in the medullary mechanism.

Now it was shown by an ingenious experiment of Flourens (1842) that there are no cells higher than the medulla which are sensitive to changes in the concentration of the blood gases in such a way as to originate respiratory impulses, so the question of a possible higher regulation of respiration by cells sensitive to changes in CO_2 may be dismissed.

The choice would seem to lie, then, between the second and third possibilities. That is, between the possibility that the cells of origin of these reticular fibers are activated by afferent impulses arising somewhere in the peripheral respiratory mechanism and that the efferent fibers pursue a course independent of those arising in the cells of the so-called medullary center—the "noeud vital" of Flourens, to the motor cells of the ventral horns, or that these cells of origin of the reticular fibers, activated by afferent impulses from the peripheral respiratory field, terminate in the respiratory center in the medulla and have no further independent efferent pathway. A modification of the first of these two possibilities would be to suppose that the cells of origin of the efferent reticular fibers are activated by impulses coming up from the respiratory center itself.

The experiments of Gad and Marinesco (1893) on burning the cells of the medullary center with hot glass beads would seem to have a bearing on our decision. The destruction of the circumscribed area of the lower

portion of the medulla sufficed permanently to stop all respiratory movements, either diaphragmatic or thoracic, on the same side of the body. One would conclude from these experiments, either that the cells of origin of the reticular efferent fibers are excited by impulses coming up from the respiratory center or that they have no independent pathway lying at any significant distance from the respiratory nucleus in the lower portion of the medulla. Incidentally, the observation of Flourens, that the respiratory movements of the mouth and nostrils ceased after transection above the noeud vital shows that there are ascending fibers from the medullary center to the nuclei of the seventh and fifth cranial nerves. It would seem improbable therefore, that the fibers Allen describes have any efferent path independent of the respiratory center in the bulb if they are to be regarded as of very great importance in the ordinary movements of respiration. It would seem more logical to regard them as being associated with some extraneous movements of the thorax and diaphragm such as those accompanying vocalization.

In this connection, one may cite the case mentioned by Hughlings Jackson (1898) in which strictly respiratory movements of the thorax persisted, but the voluntary control of the thoracic movements was lost. So far as the relation of the corpora quadrigemina to the respiratory movements is concerned, it seems more logical to suppose that the afferent impulses coming in over the dorsal roots of the spinal nerves pass up as high as the corpora quadrigemina and there come into relation with cells of origin of afferent fibers to the respiratory center itself. It seems probable that in the cartilaginous fishes, the mesencephalic nucleus of the fifth cranial is one central afferent station (Springer, 1928) for the control of respiratory movements of the mandible. Some mechanism of correlation of the activity of the bulbar respiratory center and the motor fibers of the V, VII and IX cranial nerves seems to be established in Selachians. Judging from what we know of the general deportment of the cells of the bulbar center, the access to it of descending impulses from somewhere in the peripheral field of the fifth cranial nerve seems probable. With the fading significance of the facial and mandibular musculature in respiratory movements in vertebrate phylogeny, the impulses passing from the collicular region down to the bulbar respiratory center appear to have become associated more closely with afferent impulses from the intercostal muscles.

Summarizing briefly, it would seem that afferent impulses from the intercostal muscles come first to cells in the ventral horns of the thoracic region, probably over intercalated neurones, and second, to some central station in the collicular regions. From the collicular central stations, descending fibers pass to the bulbar center wherein the impulses over these descending fibers are summed with the changes in concentration of the gases of the blood, and efferent impulses arising in the bulbar center pass

down the spinal cord, probably over intercalated neurones, to the ventral horn cells, wherein they are summed up with the afferent impulses coming in over the dorsal roots. It would seem that the fibers described by Allen are part of some other system.

CONCLUSIONS

1. Ontogenetic development of the vagus-phrenic portion of the respiratory mechanism has been completed at birth and is functionally active from that time onward.
2. A closer relationship between the vagus and phrenic nerves in respiratory regulation than between the vagus and dorsal spinal nerve roots is demonstrated.
3. Development of the mechanism for adequate thoracic respiration is not completed before the end of the third week after birth in the kitten, as is shown by its lack of ability to compensate for failure of diaphragmatic respiration.
4. The functional relationship of the nuclei in the corpora quadrigemina to the costal respiratory mechanism is not established earlier than ten days after birth and possibly later.

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ELECTRICAL MEASUREMENTS OF NEUROMUSCULAR STATES DURING MENTAL ACTIVITIES

III. Visual Imagination and Recollection

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It is a familiar fact in man as in all vertebrates that the eyes tend to be directed, by contraction of the striated muscles, toward the object which is seen. But whether specific eye-movements are associated with specific mental activities has never been adequately investigated (cf. Jacobson, 1929, chap. X). The present aim is to test whether the recti muscles at the moment of visual imagination and recollection direct the eye-balls and so take active part. Electrical measurements are preferred to mechanical because the eye-lids are kept closed. Owing to restrictions of space, only the major points of methods and results can here be stated.

Methods. The electrodes used in most of the present tests have been previously described. Thermostatic regulation was omitted. One cell with electrode was generally attached above the medial portion of the right orbital ridge and the other over the right mastoid bone. In the other tests, the electrodes generally consisted of platinum foil about 1×2 cm., wrapped in cotton previously immersed in 0.9 per cent NaCl solution. Evaporation was prevented by a covering of "silkloid" surgical dressing, attached to the skin by gummed paper.

The aim in connecting one electrode near the orbital ridge and another behind the ear is so far as possible to record monophasic action-potentials from the ocular muscles. But in a few experiments the second cell was attached just lateral to the right orbital ridge, since this arrangement tended to yield more marked galvanometric deflections.

It is necessary first to determine how to distinguish between records due to actual eye-movements from those due to convergence, frowning and winking,—in order later to be able to intrepret the tracings secured during visual imagination.

Eye-ball movements. For each test, after the electrodes have been set in place on the skin, the subject is instructed to relax his eye muscles; but later, upon hearing the first signal, he is to look slightly to the right, while upon hearing the second signal, he is to return to relaxation. Subsequently records are made similarly for looking slightly to the left, looking slightly

up, and looking slightly down. In order to favor the absence of undesired eye-movements, the eye-lids are closed during all of the present investigations.

The tracing of the string shadow during relaxation with subjects previously trained to relax their eyes is on the whole fairly horizontal. To be sure, deviations V'_m (rippling and other irregularities) are more frequent than during relaxation recorded with electrodes attached to the limbs. Apparently the eyes are not so readily relaxed as the limbs.

With a particular subject and a particular location of the electrodes, eye-movement in one direction is recorded as a photographic pattern, clearly distinctive from the patterns recorded during looking in any other direction. Typical examples are shown in figures 1, 2 and 5.

As will be seen in these figures, the electrical effects during eye-movements, as recorded by the present apparatus, do not characteristically include changes in the length of the string vibrations, V°_{2m}. Accordingly, the present article, unlike the foregoing ones (1930 a, b), will be concerned chiefly with changes in V'_m rather in V°_{2m}. An accurate quantitative expression for the patterns could be made with the integraph in terms of areas, but for the present purposes such measurements are unnecessary. Instead, the maximum ordinate of the pattern or other deviation from the baseline during each period of mental activity and each control period is measured. This value, expressed in microvolts, is known as V'_m, in agreement with the definition of this function previously given. Sometimes the entire baseline, previous to the first signal, lies above or below the line of zero potential. Nevertheless, in the present as in the preceding tests, it is convenient to measure V'_m from the baseline.

Under heading A in table 1 are shown the average magnitudes of the electrical changes accompanying eye-movement in each direction. The microvoltages for eye-movements are only approximately ascertained in the present investigations because the string shadow frequently shoots off the field so that the maximum excursion is not recorded. Additional errors or inadequacies of the present method for obtaining absolute values in the tissues have been previously stressed (Jacobson, 1930a). In this connection it should also be noted that the electrodes are considerably removed from the sources of potential differences in the recti muscles.

The average of the maximal values for the various eye-movements varies respectively for the five subjects from about 88+ microvolts to about 137+ microvolts. The plus sign indicates that in some cases the excursion of the string shadow exceeds the limits of the photographic paper.

For four out of five subjects the average microvoltage of V'_m during eye-movements is at least 400 to 500 per cent of that during relaxation. For the remaining subject, the results during eye-movements are approximately like those for the other subjects; but V'_m during relaxation averages

TABLE 1

Potential differences between electrodes attached in ocular region

SUBJECT	INSTRUCTION	NUMBER OF TESTS	Results of tests			V'_m						V°_{2m}					
						During relaxation			During test period			During relaxation			During test period		
			+	−	?	Minimum	Maximum	Average	Minimum	Maximum	Average	Minimum	Maximum	Average	Minimum	Maximum	Average
A. During eye-ball movements																	
B. R.	Look right, left, up, down	44	43	1	0	8	80	27	29	189+	137+	5	30	16	5	60	27
L. G.	Look right, left, up, down	20	20	0	0	9	59	21	21	210+	89+	1	17	6	2	38	9
B. L.	Look right, left, up, down	4	4	0	0	42	155	71	92	126+	105+	5	30	17	5	30	17
B. J.	Look right, left, up, down	4	4	0	0	17	42	21	34	147+	88+	9	17	13	9	17	13
B. E.	Look right, left, up, down	4	4	0	0	13	29	21	34	168+	98+	5	17	9	5	17	9
B. During visual imagination																	
B. R.	Imagine	47	45	2	0	8	170	36	55	240+	122+	5	84	16	5	84	21
B. R.	Controls	12	0	12	0	8	170	34	8	170	34	5	21	11	5	21	11
B. E.	Imagine	9	9	0	0	13	42	19	13	118	80	5	17	9	5	17	9
B. E.	Controls	3	0	3	0	13	42	19	13	42	19	5	17	9	5	17	9
B. L.	Imagine	30	30	0	0	13	90	45	21	170+	115+	9	30	17	10	42	21
B. L.	Controls	6	0	6	0	13	90	45	13	90	45	9	30	17	9	30	17
L. G.	Imagine	12	10	0	2	14	60	32	23	147	70	2	13	8	2	13	8
L. G.	Controls	4	0	3	1	25	60	35	25	60	35	4	17	9	4	17	9
B. J.	Imagine	14	13	0	1	13	71	44	38	139	86	1	17	12	1	17	12
B. J.	Controls	3	0	2	1	13	71	44	13	71	44	1	17	12	1	17	12
C. During visual recollection																	
B. R.	Recall	31	29	0	2	7	103	32	29	138	84	8	55	21	8	139	34
B. R.	Controls	6	0	6	0	7	103	32	7	103	32	8	55	21	8	55	21
B. L.	Recall	24	24	0	0	20	80	49	40	210	97	10	38	22	15	85	39
B. L.	Controls	6	0	6	0	20	80	49	20	80	49	10	38	22	10	38	22
L. G.	Recall	9	9	0	0	21	44	34	21	81	45	9	27	19	9	27	19
L. G.	Controls	2	0	2	0	21	44	34	21	44	34	9	27	19	9	27	19
B. E.	Recall	1	1	0	0	29	34	32	118	118	118	5	13	7	5	13	7

This table is an abstract from three tables, corresponding to the headings A, B, C. Under each heading, for each subject, the least value found in *any* test is set down as the minimum, the greatest value is set down as the maximum, while the average of average values is here set down as the average. In the second column the character of the instruction is indicated, but the complete statement of what is imagined or recalled will be found in the text. The total number of tests is shown in column 3.

about 260 per cent or more of that for the other subjects, indicating that she does not succeed in relaxing her eyes as well as the other subjects when instructed to do so. A very slight flutter of her eye-lids could frequently be observed.

During eye-movements as compared with relaxation, there is no change in V°_{2m} except for two subjects, for whom the average increase is respectively 69 and 50 per cent. Without doubt, from the work of many previous investigators as well as my own, action-potentials, occurring frequently per second, occur in the tetanic contractions of all striated muscles, including the recti muscles during eye-movement; therefore the present failure to record action-potentials in the form of increased linear deflections, V°_{2m}, must be due to the relatively great distance of the place of attachment of the electrode from the muscles. Decreasing this distance by placing the electrode near to the muscle whose action-currents are to be recorded is found to increase the magnitude of V'_m. For example, this results for upward movements if the electrode is above the eye and for downward movements if the electrode is below. Probably, therefore, the electrical changes herein called V'_m result from numerous action-potentials preponderating in a particular electrical direction.

Reaction time is measured as the interval from the beginning of the first signal sound to the beginning of V'_m: In 49 instances, with one exception, for all subjects, the reaction time is under one second. The average for all instances is 0.5 second or under. It is therefore evident, following considerations previously mentioned, that the electrical phenomena here studied are not the psychogalvanic reflex.

Results are called "+" (column 4) if V'_m during the test period clearly exceeds the corresponding value during relaxation.

In the columns headed V'_m are shown the microvoltage values of the maximum ordinates of the changes in curve during relaxation and during the test periods. (Minimum and maximum values, being selected from the total range of series of tests with each subject, will of course vary much more than if selected from the results for a particular set-up on any given date.) Values here set down as "average" correspond with what in preceding tables has been termed "average of averages."

In the columns headed V°_{2m} are the microvoltage values of string vibrations (occurring frequently per second). As will be seen, little or no change characteristically takes place with the present electrical connections during test periods as compared with rest.

A. *During eye-ball movements*, the average microvoltage of V'_m is at least 400 to 500 per cent as great as that during relaxation. See text for discussion of the exceptional case, B. L.

B. *During visual imagination*, as compared with relaxation, the values of V'_m are seen to be markedly increased (107 out of 112 cases). V'_m is not increased during the tests termed "controls," which follow the instruction "not to bother to imagine."

C. *During visual recollection*, compared with rest, there is similar marked increase of V'_m (63 out of 65 cases); but no such increase occurs during the control tests.

Relaxation time is measured as the interval from the beginning of the second signal sound to the approximately complete disappearance of the change in curve, V'_m. The photographic records disclose that the subject frequently (21 out of 49 cases) relaxes his eye-muscles before the signal to relax has been given. In the instances where relaxation begins after the second signal, the average relaxation time for each of the four subjects varies from 2.4 to 4.0 seconds.

Convergence. Convergence is produced by having the subject look off in the distance and then, upon prearranged signal, focus on a point as marked by some object placed at a fixed distance (e.g., 24 cm.) from him in his line of vision. With the glass electrodes and attachments as above described, relatively little change in the records occurs as compared with the period of relaxation,—excepting such change as can generally be ascribed to eye-movements, right, left, up and down, following the signal to converge.

Eye-lids and adjacent muscles. During contraction of the orbicular muscles, following the instruction to wink or to close the eyes tightly, increase of V°_{2m} occurs in 16 out of 19 instances, averaging about 133, 233 and 333 per cent of the value during relaxation respectively for the three subjects tested. But increase in V'_m occurs in only 10 out of the 19 instances, and the increased value, when present, averages only 165 per cent of the value during relaxation for all subjects. In summary, with the present electrical connections, eye-ball movements are distinguished by increase of V'_m with little or no increase of V°_{2m}; while as a rule contractions of the orbicular muscles show marked increase of V°_{2m}, with or without marked increase of V'_m. Furthermore, eye-ball movements are distinguished (from orbicular and other adjacent muscular contraction) by the occurrence of a different photographic pattern for each direction.

Visual imagination. Previous to a particular series of tests, the subject has been instructed to lie with eye-lids closed and ocular muscles relaxed until he hears the first signal, when, for instance, he is to "imagine Eiffel Tower in Paris." Upon hearing the second signal, he is to relax all ocular tensions. After intervals permitted for relaxation, the above-mentioned set of signals is several times repeated. As will be evident, this instruction to imagine is likely to result in the subject's visualizing the object mentioned. This result can be confirmed by his report.[1]

The character of potentials from the eye region during visual imagination: During a period of general relaxation, when the galvanometer string is vibrating slightly and uniformly, recording a fairly horizontal band on the photographic paper, the signal is given which will incite the subject to visual imagination. Generally within a fraction of a second, the string shoots forth and back or

[1] Other instructions to imagine include a sky-rocket shooting up, the statue of Liberty, Denver, a circle, the point of a pin, a flower fluttering in the wind, London Bridge, the lake, a motor car passing.

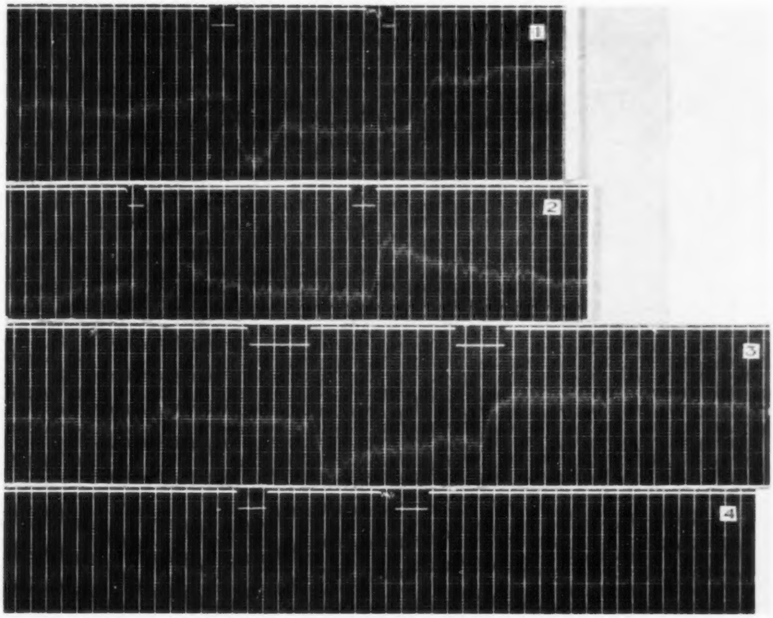


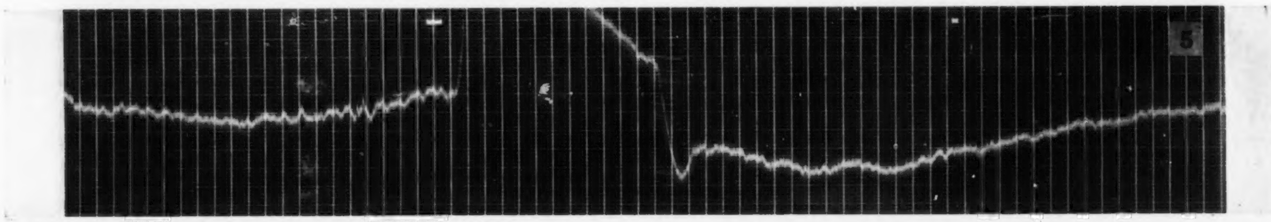
Fig. 1. *Photographic record of potential differences during eye-movement,—looking upward.* Platinum foil electrodes, 2.5 cm. square, covered with cotton moistened with 0.9 per cent NaCl solution. One electrode is placed 1 cm. above the right orbital ridge with its midline lying above the outer boundary of the inner third of the eye. The other is placed 1 cm. to the right of the external canthus. Vertical lines indicate time intervals $\frac{1}{5}$ second. Distance between parallel horizontal lines is 1 mm. = 4.2×10^{-6} volts.

The subject is instructed to look upward when he hears the signal. Previous to this sound, the eyes have been fairly quiet, as shown by the fairly horizontal tracing of the string shadow. About 0.2 second after the signal begins, the string shadow is deflected approximately 2 cm. and proceeds to trace a pattern on the photograph characteristic for this subject with this set-up for looking upward. About 0.6 second after the beginning of the second signal, the eyes begin to return to a quiet state. Subject B. E.

Fig. 2. *Photographic record of potential differences during eye-movement,—looking left.* Conditions the same as for figure 1. The string here traces a pattern on the photograph characteristic for this subject with this set-up for looking to the left, and showing obvious differences from the pattern shown in figure 1.

Fig. 3. *Photographic record of potential differences during visual imagination.* All conditions the same as for figure 1. But the subject has here been instructed, "When the signal comes, imagine Eiffel Tower in Paris." Before this signal the string has been fairly quiet. About 0.8 second after the beginning of this signal, the string is deflected approximately 2 cm., and proceeds to trace a pattern on the photograph characteristic for this subject with this set-up for looking upward. Note that figure 3 strikingly resembles figure 1.

Fig. 4. *Control record for tests on visual imagination.* This photograph was taken soon after that shown in figure 3, under the same conditions. The instruction is, "When the signal comes this time, do not bother to imagine. Upon hearing the second signal, if any ocular tensions should be present, relax them in the usual manner." As will be seen, the results are negative: the eyes remain practically quiet throughout the entire test period.



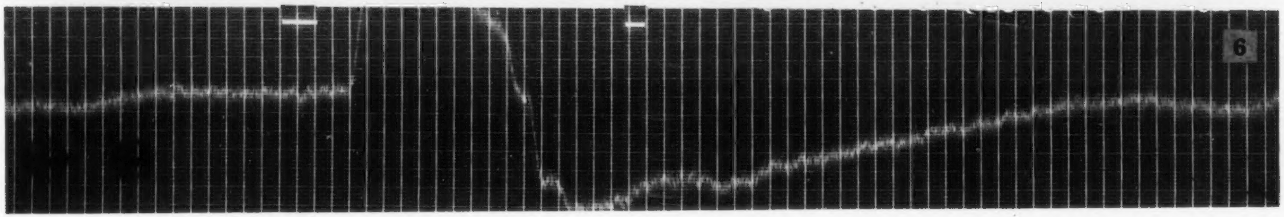


Fig. 5. *Photographic record of potential differences during eye-movement,—looking right.* Electrodes as in figure 5, in article I (1930a). One is placed about 2.5 cm. above the inner canthus of the right eye, that is, about 1 cm. above the orbital ridge. The other electrode is placed just to the right of the right eye, its lower edge on a level with the external canthus, 1 cm. from the orbital ridge. Other conditions same as for figure 1.

About 0.4 second after the beginning of the signal to look to the right, the string shows a marked deflection exceeding 2.5 cm., and passing off the film. The pattern traced is characteristic for this subject with this set-up for looking to the right. Subject B. R.

Fig. 6. *Photographic record of potential differences during visual recollection.* Conditions as for figure 5. But the instruction has been, "When the signal comes, recall this morning's newspaper." Before this signal the string has been fairly quiet. About 0.8 second after the beginning of the signal, the string shows a marked deflection exceeding 2.5 cm. and passing off the film. The string then proceeds to trace a pattern on the photograph characteristic for this subject with this set-up for looking to the right. Note that figure 6 strikingly resembles figure 5.

alternately for fractions of a second or more, producing deviations from the horizontal on the photographs. These deviations cease soon after the signal is given to relax any muscular tensions present (fig. 3).

If the room is not too dark, the experimentor generally can observe that the closed eye-lids of the subject appear quiet during extreme relaxation, whereas a slight movement or convergence of the eye-balls as a rule can be detected at the moment of visual imagination (Jacobson, 1925). Likewise the photographic records for visual imagination show patterns resembling those above-described for eye-movements. This is clearly illustrated in figure 3 where the pattern for imagining Eiffel Tower is practically identical with the pattern of the same subject for looking upward (fig. 1). Evidently in imagining the tower, the subject's eye-balls move upward, somewhat as they would upon actually seeing a tower. Correspondingly he reports that in imagining he looks from the base to the top of the tower.

Under heading B in table 1 are shown the magnitudes of electrical changes recorded during visual imagination. For all subjects together, positive results in the form of marked changes in V'_m during imagination as compared with relaxation occur in over 95 per cent of the cases (107/112). Additional control tests, interspersed among the tests of imagination, follow the instruction, "This time when the first signal comes, do not bother to imagine." Of 28 such control tests with all subjects, 26 proved negative (fig. 4).

The microvoltages (V'_m) recorded for visual imagination (table 1, B), while smaller for four out of five subjects than for eye-movements (table 1, A), are yet sufficiently near the latter in magnitude to indicate that actual eye-movements commonly occur during visual imagination.

The above results apparently suggest that the imagined object is seen somewhere in the visual field. Likewise, reports carefully elicited from the trained subject indicate that in his experience the object visualized always appears as in some location, definite or spread out, in his visual field. Upon seeing an object in imagination, he reports, there are sensations from the recti muscles, similar to those present when actually looking with open eyes. Needless to say, directions of objects so imagined do not always correspond to actual locations of the same objects; for instance, the subject may imagine the lake as in the west but his eye-movement may be toward the south.

Reaction and relaxation times. For all subjects the reaction time for visual imagination is generally (83 out of 96 instances) under one second. The average value is 0.53 second. This low figure again indicates that we are not dealing with the psychogalvanic reflex.

In 71 out of 93 cases where relaxation occurs after the beginning of the second signal, relaxation, on the average, is fairly complete for the five subjects within 3.8 to 5.8 seconds.

Visual recollection. Under the same conditions, tests were made with four subjects to determine whether the results observed for visual imagination hold similarly for visual recollection. Instructions are, "recall this morning's newspaper," "recall Denver, Colorado," "recall this morning's breakfast table," and so forth.

Examination of the photographic records for recollection (fig. 5) discloses patterns similar to those for eye-movements and for visual imagination. The magnitudes of the electrical changes recorded during visual recollection are shown in table 1 under heading C. For all subjects the results are positive in over 97 per cent of the cases (63 out of 65). A somewhat lesser average microvoltage for three out of four subjects indicates that eye-movements are generally less pronounced for visual recollection than for visual imagination.

The further findings for visual recollection do not differ markedly from those described for imagination.

CONCLUSIONS

1. Electrical records can be secured characteristic respectively of looking to the right, left, up and down. These can generally be distinguished from frowning, winking, and convergence.

2. During visual imagination and recollection electrical records are secured from the ocular muscles, producing photographic patterns like those following instructions to look in one direction or another. As shown by these records, eye-movements characteristically occur during visual imagination and recollection.

When the trained subject is requested to relax his eye-muscles, the curve becomes relatively horizontal and straight, characteristic of relaxation, and the visual imagination or recollection disappears, according to his report. Likewise, during control periods, when the subject has been instructed to keep his eye-muscles relaxed, he reports the absence of visual imagination or recollection.

3. Reaction and relaxation times, except for minor differences, tally for tests following the instruction to imagine or recollect as compared with the instruction to look in a particular direction.

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ELECTRICAL MEASUREMENTS OF NEUROMUSCULAR STATES DURING MENTAL ACTIVITIES

IV. Evidence of Contraction of Specific Muscles During Imagination

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During imagination and recollection, as tested with electrodes attached in the region of muscles which contract in the actual performance of the act imagined or recalled, increased vibrations of the amplified galvanometer string show the presence of potential differences, called action-potentials (Jacobson, 1930a, b). We now inquire whether under these conditions the presence or absence of action-potentials signifies the corresponding presence or absence of muscular contraction.

Methods. *To record arm movement*, the string recorder of Einthoven and Hugenholtz (1918) was employed at first. The lever arm of bamboo was prolonged to yield a magnification of about 40 to 100, as desired, but tended to develop a period of its own, with the disadvantage that slight physiological movements might be obscured. Therefore a lever mounted on a shaft with jewel bearings and provided with a damping spring was preferred. Such an assembly was procured from a manufacturer of voltmeters, following a suggestion by Mr. A. B. Shaw.

The right forearm, with radial side uppermost, rests firmly in a well supported copper V. At the lowermost portion of the radius, a phosphor-bronze cuff is firmly bound. A short vertical strip of metal, soldered to the uppermost portion of this cuff, carries a horizontal screw on which turns a round-nut and lock-nut. From an attachment in a groove in the round-nut, an inelastic fiber of fine silk extends directly upward to a point on the short lever-arm. Adjustments of the position of the lever-arms can be made by turning the round-nut and by manipulating a worm-gear supporting the lever assembly. Accordingly, the lever is so placed that the upper end of its longer arm, when vertical or approximately so, casts a shadow upon the moving photographic paper.

To secure electrical records, the circuit is arranged as above described, excepting that two platinum iridium needle electrodes are used, -25 gauge, $\frac{3}{4}$ inch long. One electrode is inserted in the biceps, approximately

vertically, about 6 or 8 cm. above the level of the tip of the olecranon process. The wire soldered to this needle is connected to the amplifier input terminal marked "+" in figure 1 of article I (Jacobson, 1930a). The other needle is inserted under the skin over the coronoid fossa and is connected by a wire to the negative input terminal. These electrodes have two advantages: 1. They permit rapid attachment to the subject. 2. They are kept at approximately the same temperature by the tissues.

Throughout the present investigation the galvanometer string is (approximately) critically damped.

To incite imagination on the part of the subject, the same methods are followed as those previously described. The instructions are "When the signal comes, imagine steadily bending the right arm," or "When the signal comes, imagine lifting a ten-pound weight held in the right hand." (With one subject similar instructions were given for imagining extending the right arm, but the electrodes were shifted,—one in the right triceps muscle and the other under the skin over the olecranon process.)

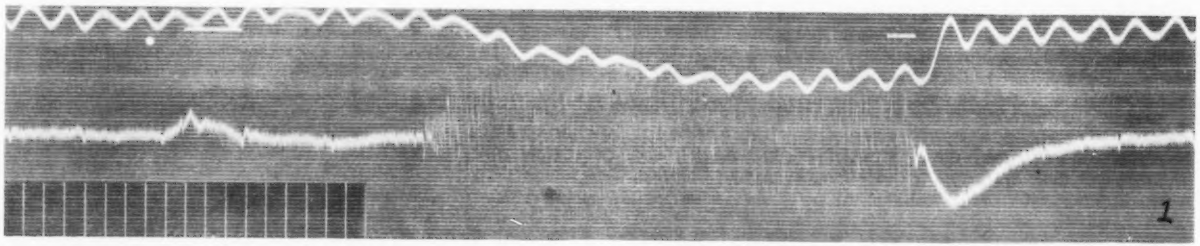
For control tests, the periods of rest are recorded prior to the first signal and subsequent to the second signal. Special control tests follow the instruction, "This time when the signal comes, imagine steadiy bending the left arm" (control 2), or "This time when the signal comes, imagine lifting a ten-pound weight with the left arm" (control 2).

Four subjects are used in the present investigation, two (B. E. and B. L.) trained to relax, and two (I. G. and A. T.) not so trained. One subject, A. T., is here used for the first time. There are disadvantages in using untrained subjects, as previously noted, namely, the frequent failure to relax promptly after the imagining; but the use of untrained subjects permits us to test whether the objective results uniformly recorded during mental activity for trained subjects are artifacts due to training.

Results. *Following the signal for the subject to imagine steadily bending his right arm or to imagine lifting a ten-pound weight in the same manner, the lever records a flexion of the arm, generally of microscopic extent.* Soon after the second signal, which signifies that the subject is to relax any muscular tensions present, there occurs a sudden return of the lever to the position it had while the arm was at rest. Likewise following the first signal and generally terminating soon after the second signal, there occur the increased vibrations of the string, V^0_{2m}, which have been described in the foregoing articles as characteristic of muscular imagination (fig. 1).

But in the control tests 2, where the subject is to imagine bending the left arm or to imagine lifting the weight with that arm, no microscopic flexion of the right arm and no action-potentials from the biceps region are recorded (fig. 2).

When a lever magnifying 40-80 fold is attached as above described, it is seen that the right arm is not motionless even during complete muscular



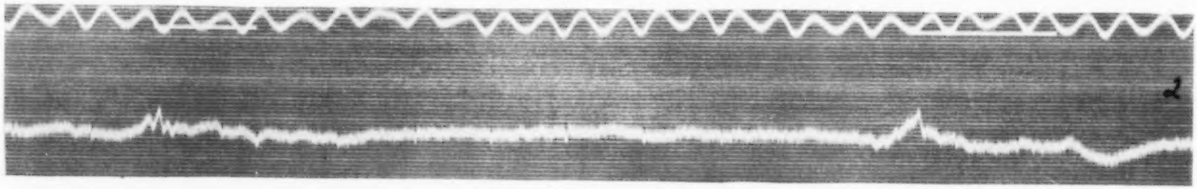


Fig. 1. The first signal (see top of photograph to the left) means, "Imagine bending your right arm," while the second signal means, "Relax any muscular tension present." Upper tracing is from shadow of lever arm; downward excursion indicates arm flexion magnified 78×. Lower tracing is from shadow of galvanometer string; downward deflexion indicates negative potential in the electrode in contact with the biceps muscle, compared with the other electrode. String critically damped. 1 mm. = 2.2×10^{-6} volts. Vertical markings in left lower corner indicate time in $\frac{1}{5}$ second.

About 2.6 seconds after the onset of the first signal, arm flexion sets in, accompanied by greatly increased string vibrations. The flexion and the action-potentials cease promptly after the signal to relax.

Undulations in upper tracing indicate arm-movements due to the pulse. Subject A. T., not trained to relax.

Fig. 2. Conditions as for figure 1, except that the instruction denoted by the first signal is, "Imagine bending the left arm." No flexion now occurs in the right arm and action-potentials likewise are absent from the right biceps region.

relaxation. Rhythmical rises and falls continually occur due to pulse and respiration. The extent of motion due to the pulse may be about $\frac{1}{16}$mm. or more, while the slower changes of position due to respiration may be several times as great. In consequence of these rhythmical motions and their frequent variations as recorded on the photographs, the precise instant at which the microscopic movement begins is not so readily determined as would be the case if the lever traced a perfectly straight line when the arm is completely relaxed. Therefore, in order to allow time for the microscopic flexion to appear clearly, the experimentor generally permitted more time to elapse between the first and second signals than in the tests recounted in previous articles,—namely, 5 to 10 seconds.

Results of the present investigation are summarized in table 1. In all of 59 tests during imagination with the four subjects, microscopic flexion of the right arm is recorded as well as action-potentials from the right biceps region. Of 19 control tests, when the subject is instructed to imagine bending the left arm, the results recorded from the right arm are mechanically and electrically negative.

During the periods when the subjects are instructed to relax, the string vibrates slightly and uniformly for the two trained subjects, but for the two untrained subjects there are sometimes fairly prolonged intervals when the vibration is markedly irregular and large in extent. During such intervals, the experimentor sometimes instructed the subject to relax and this instruction was frequently followed by subsidence of the string vibrations. In other respects the results for the two untrained subjects were the same as for the others, showing that the electrical and mechanical records found to be characteristic of mental activity are not artificially introduced through the effects of training.

During imagination, the average microvoltage of V_{2m} is about 1200 to 1700 per cent of that during relaxation for the four subjects. The range of average figures varies from 50 to 83 microvolts for the four subjects. These figures are somewhat higher than those recorded for similar tests in the preceding articles,—and this is due, at least in part, to the prolonged period of imagination employed in the present tests; for inspection of individual records discloses in many instances a marked increase in microvoltage in the latter portion of the record.

Records of deviations of curve, V'_m, are omitted from table 1 because the values do not markedly differ on the average during imagination as compared with relaxation. This is in harmony with our previous conclusion that the variations V'_m are not here characteristic of imagination. Possibly the relative infrequency of variations V'_m during the present tests as compared with those of previous articles is because the needle electrodes penetrate the muscle tissue, whereas the glass-cell electrodes (shown in fig. 5 of article I) cover a relatively large area of skin.

TABLE 1

Electrical and mechanical measurements during imagination

Instructions indicated in column 1 are as follows: (a) "Imagine steadily bending the right arm"; (a′) "Imagine steadily bending the left arm" (control 2); (b) "Imagine steadily lifting a ten-pound weight with the right arm"; (b′) "Imagine steadily lifting a ten-pound weight with the left arm" (control 2); (c) "Imagine steadily extending the right arm"; (c′) "Imagine steadily extending the left arm" (control 2).

One electrode (+) is in the biceps, and the other is over the coronoid fossa; excepting for tests c and c′, where they are respectively in the triceps and under the skin over the olecranon process.

Action-potentials, V°_{2m}, are present in all the cases of imagining and absent in all of the control tests, as is noted in columns 3 and 4. The microvoltage of string vibrations during relaxation is recorded in the next three columns. Values recorded as minimum, maximum and average represent all the tests of a particular record, rather than a single test. This applies likewise to the next three columns.

During imagination, the average microvoltage is about 1200 to 1700 per cent of that during relaxation for the four subjects (compare columns 10 and 7).

In all instances, following the instruction to imagine, a microscopic bending of the arm takes place (column 11). In all control tests, such movement is absent (column 12). The maximal extent of such movement in millimeters measured at the lower end of the radius, is stated in column 13. The time in seconds from the beginning of the first to the beginning of the second signal in each test is stated in column 14. In column 15 are shown the number of cases in which the mechanogram shows a practically steady rise during virtually the whole of this time period. For these cases, the average velocity is shown in the last column. As will be seen, this ranges from about 0.004 mm. per sec. to 0.1 mm. per sec. in the tests with the various subjects.

INSTRUCTION	NUMBER OF TESTS	ELECTRICAL RECORD (V°_{2m}) Results of tests		Microvolts during relaxation			Microvolts during test periods			ARM FLEXION				Velocity	
		+	−	Minimum	Maximum	Average	Minimum	Maximum	Average	Present	Absent	Maximal extent (average)	Duration (average)	Cases constant	Per second
Subject I. G.															
												mm.	*seconds*		*mm.*
Imagine (a)	7	7	0	4	9	7	11	132	88	7	0	0.32	3.4	7	0.10
Control (a′)	3	0	3	4	9	7	4	9	7	0	3	0.00			
Imagine (b)	6	6	0	4	9	7	13	121	77	6	0	0.25	5.8	6	0.05
Control (b′)	2	0	2	4	9	7	4	9	7	0	2	0.00			
Total imagining	13	13	0							13	0			13	
Total controls	5	0	5							0	5				
Average of averages for imagining						7			83			0.29	4.5		0.08

TABLE 1—*Concluded*

INSTRUCTION	NUMBER OF TESTS	ELECTRICAL RECORD ($V^{\circ}{}_{2m}$)								ARM FLEXION					
		Results of tests		Microvolts during relaxation			Microvolts during test periods							Velocity	
		+	−	Minimum	Maximum	Average	Minimum	Maximum	Average	Present	Absent	Maximal extent (average)	Duration (average)	Cases constant	Per second
Subject B. E.															
												mm.	*seconds*		*mm.*
Imagine (a)	5	5	0	2	4	3	13	70	44	5	0	0.26	5.6	1	0.05
Control (a′)	1	0	1	2	4	3	2	4	3	0	1	0.00			
Imagine (b)	6	6	0	2	4	3	13	99	55	6	0	0.29	5.2	5	0.07
Control (b′)	1	0	1	2	4	3	2	4	3	0	1	0.00			
Total imagining	11	11	0							11	0			6	
Total controls	2	0	2							0	2				
Average of averages for imagining						3			50			0.28	5.4		0.06
Subject B. L.															
Imagine (a)	1	1	0	3	3	3	7	77	33	1	0	0.07	5.8	1	0.01
Control (a′)	1	0	1	3	3	3	3	3	3	0	1	0.00			
Imagine (b)	4	4	0	3	3	3	7	110	66	4	0	0.15	7.1	4	0.02
Control (b′)	1	0	1	3	3	3	3	3	3	0	1	0.00			
Imagine (a)	5	5	0	2	4	3	18	88	53	5	0	0.17	9.1	4	0.02
Control (a′)	2	0	2	2	4	3	2	4	3	0	2	0.00			
Imagine (b)	5	5	0	2	4	3	18	110	66	5	0	0.24	8.7	5	0.03
Control (b′)	3	0	3	2	4	3	2	4	3	0	3	0.00			
Imagine (a)	4	4	0	2	4	3	13	79	44	4	0	0.16	8.4	2	0.02
Imagine (c)	5	5	0	4	9	7	13	121	77	5	0	0.23	4.7	3	0.07
Control (c′)	2	0	2	4	9	7	4	9	7	0	2	0.00			
Total imagining	24	24	0							24	0			19	
Total controls	9	0	9							0	9				
Average of averages for imagining						4			60			0.19	7.5		0.03
Subject A. T.															
Imagine (a)	4	4	0	1	44	4	13	121	44	4	0	0.09	18.9	1	0.004
Control (a′)	1	0	1	1	44	4	1	44	4	0	1	0.00			
Imagine (b)	7	7	0	1	66	4	22	132	77	7	0	0.13	7.9	1	0.05
Control (b′)	2	0	2	1	66	4	1	66	4	0	2	0.00			
Total imagining	11	11	0							11	0			2	
Total controls	3	0	3							0	3				
Average of averages for imagining						4			65			0.12	11.9		0.027

The mechanogram indicates that the maximal excursion of the arm, averaged for each subject, varies from 0.07 mm. to 0.32 mm. In 40 of the 59 records, there is shown a practically uniform motion per second, provided that allowance be made for variations due to pulse and respiration. In these instances it is possible to determine the velocity of motion. This is found to vary from a minimum of 0.004 mm. per second to a maximum of 0.1 mm. The majority of the figures range between 0.02 and 0.05 mm. per second.

It is evidently desirable to include as many tests as possible bearing upon the question whether the subject can imagine flexing the right arm or lifting a weight with that arm without actually engaging in at lease a microscopic flexion of the right arm. The following tests are devised so as not to depend upon the reports of the subjects. (As previously said, the trained subjects all agree that in their experience it is impossible simultaneously to imagine bending the right arm and to keep it perfectly relaxed. Whether visualization can occur alone is a matter for subsequent tests.)

1. The subject is instructed, upon hearing the first signal, to imagine bending the right arm or to imagine lifting a weight with that arm. Electrodes are attached to the right biceps region in the usual manner, and the lever is also attached as above described.

In test α, the above procedure is carried out and he is instructed to "cease to imagine" when he hears the second signal.

In test β, the procedure is the same except that he is instructed to "relax any muscular tensions present in the right arm" when he hears the second signal.

The photographic records produced in *tests α* and *tests β* are found to be identical in type. They are entirely like those shown in figure 1. That is, with instructions and records as above stated the instruction to cease to imagine is followed by the same result as the instruction to completely relax muscularly. This is evidence, secured without the intervention of the report of the subject, that ceasing to imagine is, at least in part, the same process in this instance, as muscular relaxation. It supports the view that the microscopic muscular contraction is here requisite to the act of imagination.

2. The subject is instructed, upon hearing the first signal, to keep the right arm completely relaxed and simultaneously to imagine bending the right arm or to imagine lifting a weight with that arm. Mechanical and electrical attachments continue as above described.

In test A the instruction is added that if the subject finds it impossible to imagine and to relax simultaneously as above directed, he shall at any rate engage in imagination.

In test B the procedure is the same except that here the additional instruction is that if the subject finds it impossible to imagine and to relax

simultaneously as above directed, he shall at any rate keep his right arm perfectly relaxed.

The photographic records produced in tests A are entirely like those shown in figure 1, while those produced in tests B are entirely like those shown in figure 2. This indicates without the intervention of the report of the subject, but entirely in conformity with that report, that it is found impossible simultaneously to imagine bending the right arm and to keep it perfectly relaxed. Analogous results apply to imagining lifting a weight with the right arm. The photographic records indicate that the subject either imagines,—whereupon action-potentials appear along with at least a microscopic flexion,—or else, he relaxes the right arm completely, whereupon the electrical and mechanical records are negative.

The results recounted above under 1 and 2 afford additional objective evidence that in the instances studied, slight muscular contraction is requisite to the process of imagination.

Comment. In all instances when arm flexion clearly appears in the records, action-potentials from the biceps region are marked. As previously stated, passive shifts of the arm occur, due to pulse and respiration, and render it impossible to determine the precise moment of the onset and cessation of flexion. With this reservation, it seems safe to conclude that our records clearly show that arm flexion and action-potentials from the contracting muscle fibers occur simultaneously or approximately so. Owing to the obscuring just mentioned, our electrical tests seem more delicate than the mechanical ones; accordingly, in the instances where the lever system does not yield a clear record, as when contraction is very brief or slight, it would seem justified to interpolate that if action-potentials are clearly evidenced, muscle contraction is present.

Since early investigators (Matteucci, 1843; du Bois-Reymond, 1849; Bernstein, 1867; Hermann, 1877) made known that the contracting portion of a muscle at any instant is negative in potential as compared with the relaxed portion, the question has been repeatedly raised whether the mechanical and electrical changes always show corresponding simultaneous variations. Lee concluded in 1887 from observations on fatigued frog's muscles that all of the characteristics of the muscle contraction will be found in the curve of electrical tension. More recently various investigators have maintained that the frog's heart may be at a mechanical standstill while nevertheless giving off "action currents." To this, reply has been made by Einthoven and Hugenholtz (1921) and by Arbeiter (1921) that mechanical changes running parallel to electrical tensions can be observed if a sufficiently delicate string recorder is used. A similar parallelism for the frog's skeletal muscle is found by Wishart (1925). Fulton (1925) tests Beritoff's belief that the electrical response is to be separated from the mechanical response in severe fatigue, and with sufficiently sensi-

tive instruments finds them inseparable. Likewise in the investigations of Ernst (1927), the "action current" and the diminution of volume characteristic of muscular contraction are found to run parallel. Schäffer (1927) using monophasic leads from the frog heart, finds that tonus is generally accompanied by protracted deflection of the galvanometer string, but this relationship is not invariable. In summary, then, the weight of evidence has been that for every electrical variation in the response of muscle, sufficiently delicate instruments will indicate a corresponding mechanical variation.

This matter is here tested with the string galvanometer circuit and thermionic amplification. The correlation of action-potentials and actual contraction of muscle fibers is confirmed and extended to the range of low voltages recorded in the present investigation.

The electrical records of V^o_{2m} secured during imagination and recollection have been found to differ in seven points from the psychogalvanic reflex (Jacobson, 1930a). Another point is now added: 8. During imagination and recollection, as recorded in the present articles, there occurs a muscular contraction, practically simultaneous in duration with the action-potentials. Since the psychogalvanic reflex is generally conceded to be due to electrical effects depending upon elements in the skin, the proof is now conclusive that the electrical manifestations (V^o_{2m}) during imagination and recollection are not psychogalvanic phenomena.

CONCLUSIONS

1. Contraction of specific muscles takes place following the instruction to imagine an act performed with the voluntary musculature.

2. The movement usually is of microscopic extent and generally is confined within the group of muscles whose contraction would be required for the actual performance of the voluntary act.

3. Imagination of a particular act performed by some portion of the body fails to occur if at the same time the muscles of this portion are completely relaxed. This supports the view that the contraction of specific muscles is essential to the occurrence of at least certain mental activities.

4. The conclusion drawn by previous investigators that action-potentials from muscles always are associated with actual contraction is here extended to include a range of extremely low voltages.

5. Evidence is conclusive that the electrical records (V^o_{2m}) during imagination, recollection and other mental activities are not psychogalvanic reflex phenomena.

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